FORMULATION AND EVALUATION OF REPACLINIDE TRANSDERMAL PATCH

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MASTER OF PHARMACY

IN

PHARMACEUTICS

Submitted

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CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION AND EVALUATION OF REPACLINIDE TRANSDERMAL PATCH"** submitted by **P.NATARAJAN** (Reg. No: 261511152) in partial fulfillment for the award of degree of Master of Pharmacy to the Tamilnadu Dr. M.G.R Medical University, Chennai is an independent bonafide work of the candidate carried out under my guidance in the Department of Pharmaceutics, Edayathangudy.G.S Pillay College of Pharmacy during the academic year 2016-2017.

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INTRODUCTION

The term drug delivery covers a very broad range of techniques used to deliver therapeutic agents into the human body. The limitations of the most obvious and trusted drug delivery techniques, those of the ingested tablet and of the intravenous (IV) / subcutaneous (SC) / intramuscular (IM) injections, have been recognized for some time now. The former delivers drug into the blood only through hepatic system, and hence the amount in blood stream may be much lower than the amount formulated into tablet (i.e., it has low bio-availability); furthermore, liver damage is an unfortunate side effect of many soluble tableted drugs. The injection mode of delivery can be used to deliver any size of drug molecule and is versatile in this regard, but suffers from the obvious disadvantage of being invasive and painful, and the less obvious disadvantage of shortness of duration (for drugs with short half lives).

To overcome some of these limitations, other modes of delivery of drugs into the body were investigated, beginning in the early 1970s. Transdermal (through intact skin), transmucosal, transalveolar (inhalation, through lung tissue), implantable (subcutaneous and deeper implants, delivery into surrounding tissue), and injectable (IM or SC) modes of delivery have all been explored extensively over the last 25 years, with varying degrees of commercial and therapeutic success.[1,2]

The delivery of drugs using skin as the port of entry is known as transdermal administration and the drug delivery systems are known as transdermal therapeutic systems or transdermal drug delivery systems or popularly known as transdermal patches.[1]

"Transdermal therapeutic systems are defined as self contained, discrete dosage forms which, when applied to the intact skin, deliver the drug(s), through the skin, at a controlled rate to the systemic circulation."

The success of this approach is evidenced by the fact that there are currently more than 35 approved transdermal drug delivery products for the treatment of a wide variety of conditions including: hypertension, angina, motion sicknesses, and recently contraception and urinary incontinence. There are also several products in late-stage development that will further expand transdermal drug delivery usage into new therapeutic areas, including Parkinson's disease.[1,2,3]

The polymers selected for the study are Ethyl cellulose and Hydroxypropylmethyl cellulose. The drug taken up for the study is Repaglinide.

Repaglinide, an antidiabetic chemically 2-ethoxy-4-[{[(15}-3-methyl-1-[2-(piperdinyl) phenyl] butylcarbamoyl} methyl] benzolicacid ($C_{27}H_{36}N_2O_4$) It has half-life around 1 h and bioavailable.

In the present study, transdermal patches of the Repaglinide were prepared using polymers like Hydroxypropylmethyl cellulose and Ethyl cellulose, both in different combination. These transdermal patches were characterized for their physicochemical properties including drug release.

In recent years considerable attention has been focused on the development of new drug delivery systems. The conventional dosage forms such as tablets, capsules, and parenterals with the one exception of continuous intravenous infusion are inefficient because of several reasons. These dosage forms release the drug at faster rate initially leading to quick rise in blood levels and thereafter there is an exponential fall until another dose is administered. This results in peak and valley pattern of drug levels in blood. Thus, for most of the time drug concentration is either above the therapeutic level leading to adverse reaction with potent drug or below it leading to ineffectiveness. The need to minimize blood level fluctuation of drug has led to the development of controlled drug delivery system.

Recently, it is evident that the benefits of intravenous drug infusion can closely be duplicated, without its disadvantages, by using the skin as a port for drug administration, to provide continuous transdermal drug infusion in to systemic circulation.

Transdermal drug delivery systems (TDDS) are designed to deliver the drug substances from the surface of the skin through its various layers into the systemic circulation. TDDS is the delivery of drugs through the skin to achieve systemic effects. Transdermal patches control the delivery of drugs at controlled rates by employing an appropriate combination of hydrophilic and lipophillic polymers.[3,4]

Advantages [3,4]

- Prevents the variation in the absorption and metabolism associated with oral drug administration.
- Prevents the risk and inconvenience of intravenous therapy.
- Permits continuous zero-order drug administration and the use of drugs with short biological half-lives.
- Increases the bioavailability and efficacy of drugs, since it bypasses hepatic first-pass elimination.
- Provide a simple therapeutic regime, leading to good patient compliance that can be easily terminated by simple removal of the patch.
- Transdermal medication delivers a steady infusion of a drug over an extended period of time. Adverse effects or therapeutic failures frequently associated with intermittent dosing can also be avoided.
- Self-medication is possible.

Limitations [4, 5]

- ❖ The transdermal route of administration is unsuitable for drugs that irritate or sensitize the skin.
- ❖ Only potent drugs are suitable candidates for transdermal delivery due to the natural limits of drug entry imposed by the skin permeability.
- ❖ Technical difficulties are associated with the adhesion of the systems to different skin types and under various environmental conditions, and the development of rate controlling conditions.
- ❖ Drugs requiring high blood levels to achieve an effect are difficult to load into a transdermal system due to large physical amount of material required.

Factors Affecting Transdermal Permeability

The principal transport mechanism across mammalian skin is by passive diffusion, which is primarily the trans-epidermal route at steady state or through trans-appendageal route at initial non-steady state.

The factors controlling transdermal permeability are as follows:

Partition Coefficient: Drugs possessing both lipid and water solubility are favourably absorbed through the skin. Transdermal permeability coefficient shows a linear dependency on partition coefficient. A lipid/ water partition coefficient of one or greater is generally required for optimal transdermal permeability.

The partition coefficient of a drug molecule may be altered by chemical modification of its functional groups, which can be done without affecting the pharmacological activity of the drug. It has been established that membrane partition coefficient increases exponentially as the length of the lipophilic alkyl chain increases. Varying the vehicle may also alter the partition coefficient of a drug molecule.

pH condition: Solution whose pH values are very high or very low can be destructive to the skin. With moderate pH values, the flux of ionizable drugs can be affected by pH that alters the ratio of charged and uncharged species and their transdermal permeability.

Penetrant concentration: Assuming membrane limited transport, increasing the concentration of dissolved drug causes a proportional increase in flux. At concentration higher than the solubility, excess solid drug acts as a reservoir and helps to maintain a constant drug concentration for a prolonged period of time.

Transdermal Drug Penetration through Skin [6,7,8]

Physiology of Skin

Although the skin is a large and logical target for drug delivery, its basic functions limit its utility for this purpose. The skin functions mainly to protect the body from external insults (e.g., harmful substances and microorganisms) and to contain all body fluids. It must be tough, yet flexible enough to allow for movement. The lipids in our

skin serve as poor conductors of electricity and can protect us from electrical current if the need arises. The skin also helps to regulate body temperature.

There are two important layers to human skin: the epidermis and the dermis. For transdermal delivery, drugs must pass through the two sub layers of the epidermis to reach the microcirculation of the dermis. The stratum corneum (or the horny layer) is the top layer of the skin and varies in thickness from approximately 10 microns to several hundred microns, depending on the region of the body. It is composed of layers of dead, flattened keratinocytes surrounded by a lipid matrix, which together act as a brick-and-mortar system that is difficult to penetrate. The stratum corneum provides the most significant barrier to diffusion. In fact, the stratum corneum is the barrier to approximately 90% of transdermal drug applications. However, nearly all molecules penetrate it to some minimal degree. Below the stratum corneum lies the viable epidermis. This layer is about ten times as thick as the stratum corneum; however, diffusion is much faster here due to the greater degree of hydration in the living cells of the viable epidermis. It contains Langerhans' cells, which function as antigen presenting cells to the immune system. These cells are the targets for transdermal vaccine delivery. Melanocytes in the viable epidermis provide skin pigmentation. Below the epidermis lies the dermis, which is approximately one millimeter thick and 100 times the thickness of the stratum corneum. The dermis contains small vessels that distribute

drugs into the systemic circulation and to regulate temperature, a system known as the skin's microcirculation. The dermis also contains sensory neurons and a lymphatic network. The lymphatic removal of transdermally applied drugs has not been studied extensively.

There are two main pathways by which the drugs cross the skin and reach the systemic circulation. The most direct route is known as the *"transcellular pathway"*. By this route, drugs cross the skin by directly passing through both the phospholipid membranes and the cytoplasm of the dead keratinocytes that constitute the stratum corneum. Although this is the path of shortest distance, the drugs encounter significant resistance to permeation. This is because the drugs must cross the lipophilic membrane of each cell, then the hydrophilic cellular contents containing keratin, and then the phospholipid bilayer of the cell one more time. This series of steps is repeated number of times to traverse the full thickness of the stratum corneum. A few drugs have the properties to cross via this method.

The more common pathway through the skin is via the intercellular route. Drugs cross the skin by this route and pass through the small spaces between the cells of the skin, making the route more tortuous. Although the thickness of the stratum corneum is only about 20 µm, the actual diffusion path of most molecules crossing the skin is on the order of 400 µm. The 20-fold increase in the actual path of permeating molecules greatly reduces the rate of drug penetration.

A less important pathway of drug penetration is the follicular route. Hair follicles penetrate through the stratum corneum, allowing more direct access to the dermal microcirculation. However, hair follicles occupy only 1/1000 of the entire skin surface area. Consequently, very little drug crosses the skin via the follicular route.

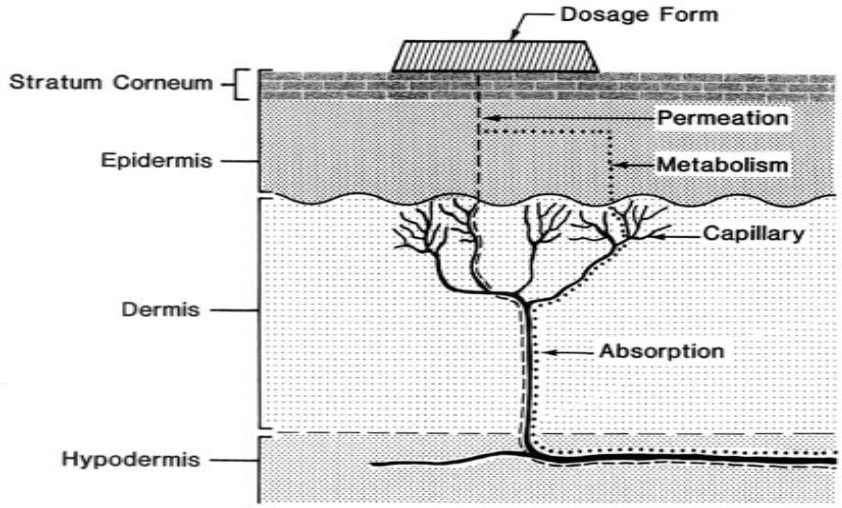


Figure 1: Diagram of skin along with transdermal patch

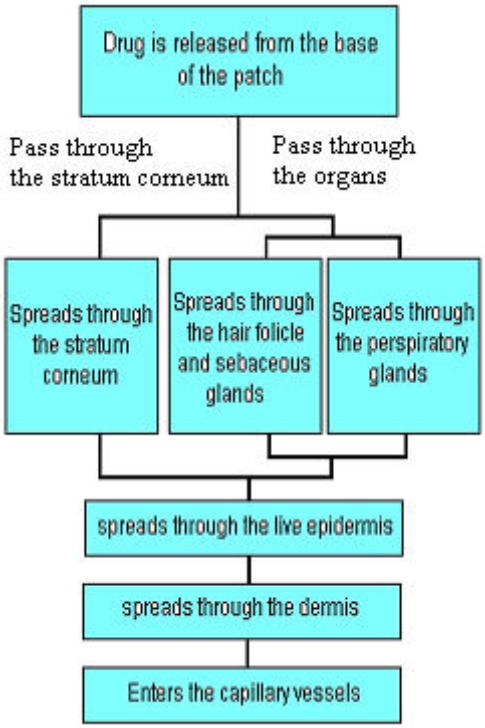


Figure 2: Flow chart of drug entry through skin into capillary vessels.

Basic Components of Transdermal Drug Delivery Systems [6,7,8]

Transdermal drug delivery systems are designed to support the passage of drug substance from the surface of skin, through its various layers, and into the systemic circulation. There are basic components of transdermal dosing system; which are those that control the rate of drug delivery to the skin. The components of devices include;

- ❖ The drug substance
- ❖ Polymer matrix
- ❖ Permeation enhancer
- ❖ Adhesives
- ❖ Backing membrane

1. The drug substance: Judicious choice of drug is critical in the successful development of a transdermal product. The important drug properties that affect its diffusion from device as well as across the skin include molecular weight, solubility, physical properties and melting point. The structure of the drug also affects the skin penetration. Diffusion of the drug in adequate amount to produce a satisfactory therapeutic effect is of prime importance. Other parameters such as skin irritation and clinical need should be considered before a drug is chosen.

The following are some of the desirable properties of a drug for transdermal delivery.

- The drug should have molecular weight less than 1000 Daltons.
- The drug should have affinity for both lipophilic and hydrophilic phases.
- The drug should have a low melting point.
- The half-life of drug should be short.
- The drug must not induce a cutaneous or allergic response.
- The drugs, which degrade in gastrointestinal tract or inactivated by hepatic first pass effect are suitable candidates for transdermal drug delivery system.

2. Polymer matrix: The polymers play a major role in transdermal drug delivery systems of drugs. The release of drug to the skin is controlled by drug free film known as rate controlling membrane. Polymers are also used in the matrix devices in which the drug is embedded in polymer matrix, which control the duration of release of drugs.

The polymers should fulfil the following requirements:

- Molecular weight, physical characteristics, and chemical functionality of the polymer must allow the diffusion of the drug substances at desirable rate.
- The polymer should be chemically non-toxic, non reactive or it should be an inert drug carrier.

- ❖ The polymer must be easy to manufacture and fabricate into the desired product. It should allow incorporation of large amount of active agent.
- ❖ The cost of the polymer should not be expensive.

The polymer controls the release of drug from the device. The polymer used should be stable, non-reactive with the drug, should allow the drug to diffuse properly and release through it. Some of the useful polymers are as follows:

Natural polymers **:** Cellulose derivatives, Zein, gelatin, gums and their derivatives etc.

Synthetic elastomers : Polybutadiene, polysiloxane, acrylonitrile, butyl rubber, neoprene etc.

Synthetic polymers **:** Polyvinyl alcohol, polyvinyl chloride, polyethylene, polyacrylate, polymethylmethacrylate etc.

3. Penetration enhancers: Penetration enhancers are molecules, which reversibly alter the barrier properties of the stratum corneum. They aid in the systemic delivery of drugs by allowing the drug to penetrate more readily to viable tissues.

They can be incorporated in transdermal formulation to obtain systemic delivery of the drug or for delivery of drugs to the deeper

layers of the skin or to achieve a given therapeutic effect with a reduced concentration of the active constituents.

Penetration enhancer should have the following properties;

- ❖ The material should be pharmacologically inert.
- ❖ It should be non-toxic, non-irritant, and have a low index of sensitization.
- ❖ The penetration enhancing action should be immediate and should have suitable duration of effect.
- ❖ The enhancer should be chemically and physically compatible with a wide range of drugs and pharmaceutical adjuncts.
- ❖ The material should spread well on the skin.
- ❖ It should be odourless, tasteless, and colourless.

They can be classified into three categories as shown in the Table 1.

Table 1: Classification of penetration enhancers

Lipophilic solvents	**Surface active agents**	**Two component system**
Dimethyl sulfoxide Dimethyl	Sodium lauryl sulphate Dodecylmethyl	Propylene glycol, oleic acid, 1-4-Butanediol

4. Adhesives

The fastening of all transdermal devices to the skin using a pressure sensitive adhesive is necessary.

An adhesive system should fulfil the following requirements.

- ❖ It should not cause irritation, sensitization or imbalance in the normal skin flora during its contact with skin.
- ❖ It should adhere to the skin aggressively.
- ❖ It should be easily removable without leaving an unwashable residue.
- ❖ It should be physically and chemically compatible with the drug, the excipients and enhancers.
- ❖ It should not affect the permeation of the drug.
- ❖ The adhesive property should not deteriorate as the drug, enhancers and excipients permeate into the adhesive.

The types of adhesives commonly used in transdermal drug delivery system are:

Rubber based adhesives: Natural gum (Karaya gum), olylisoprene, polybutene, and polyisobutelene.

Polyacrylic based : Ethyl acrylate, 2-ethylhexylacrylate, iso-octyl acrylate.

Polysiloxane based: Polydimethyl siloxane, polysilicate resins, sufloxane blends.

5. Backing membrane: It provides protection from external factors during application period. The backing layer must be impermeable to the drugs and enhancers, and as a result, it is usually impermeable to water vapour. The most commonly used backing materials are metalized polyester laminated with polyethylene, Alupoly, polyester.

Approaches Used in the Development of Transdermal Drug Delivery Systems [1, 3,5,6,8]

Four different approaches have been utilized to obtain transdermal drug delivery systems:

Membrane Permeation – Controlled Systems

In this type of system, the drug reservoir is totally encapsulated in a shallow compartment moulded from a drug-impermeable metallic plastic laminate and a rate controlling membrane, which may be micro porous or non-porous. The drug molecules are permitted to release only through the rate-controlling membrane. In the drug reservoir compartment, the drug solids are either dispersed in a solid polymer matrix or suspended in an unleachable, viscous liquid medium such as silicone fluid to form a paste like suspension.

A thin layer of drug compatible, adhesive polymer like silicone or polyacrylate adhesive may be applied to the external surface of the rate controlling membrane to achieve an intimate contact of the transdermal system and skin surface. The rate of drug release from this

type of system can be tailored by varying the polymer composition, permeability coefficient and thickness of the rate limiting membrane, and adhesive.

The major advantage of membrane permeation controlled transdermal system is the constant release of drug. However, a rare risk also exists when an accidental breakage of the rate controlling membrane can result in dose dumping or a rapid release of the entire drug content. A few examples are as follows;

1. Nitroglycerin-releasing transdermal system (Transderm-Nitro/Ciba, USA) for once a day medication in angina pectoris.
2. Scopolamine-releasing transdermal system (Transderm-Scop/Ciba, USA) for 72 h. prophylaxis of motion sickness.

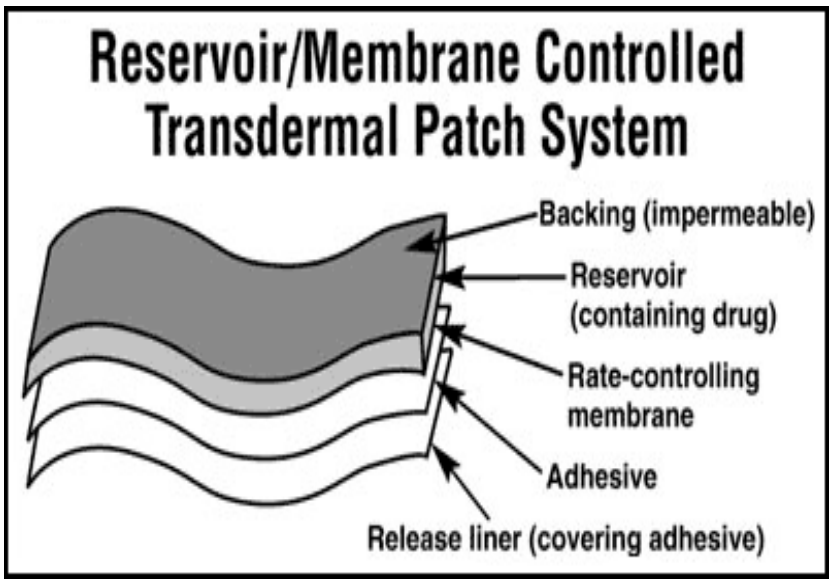


Figure 3: Reservoir/membrane controlled transdermal patch system[8]

Matrix Diffusion-Controlled Systems

In this approach, the drug reservoir is prepared by homogenously dispersing drug particles in a hydrophilic or lipophilic polymer matrix. The resultant medicated polymer is then moulded into a medicated disc with a defined surface area and controlled thickness.

The drug reservoir can be formed by dissolving drug and polymer in a common solvent followed by solvent evaporation in a mould at an elevated temperature and/or vacuum. The drug reservoir containing polymer disc is then pasted on to an occlusive base plate in a compartment fabricated from a drug impermeable plastic backing. The adhesive polymer is then spread along the circumference to form a strip of adhesive rim around the medicated disc.

The advantage of this system is the absence of dose dumping since polymer cannot rupture. One example of this type is Nitroglycerin-releasing transdermal therapeutic systems (Nitro-Dur and Nitro-Dur II / Key Pharmaceuticals, USA).

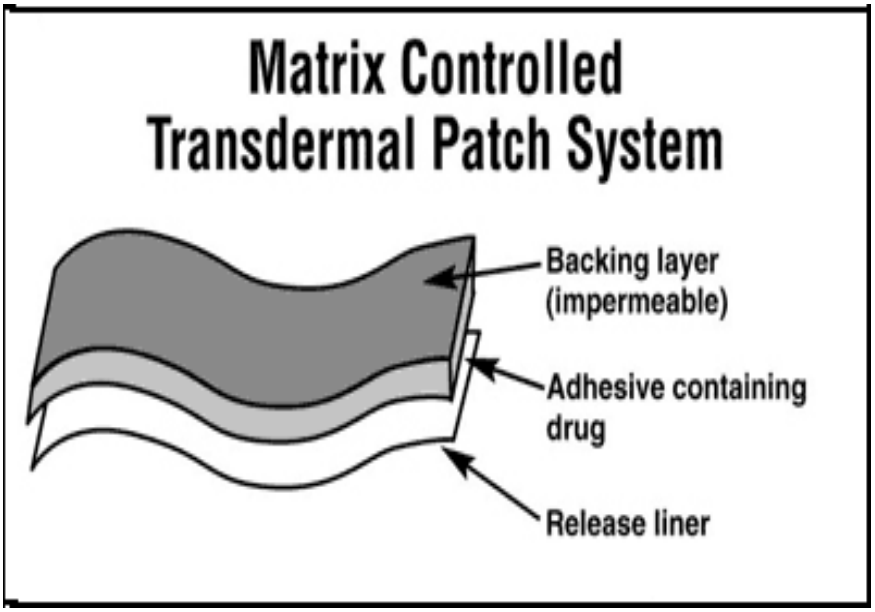


Figure 4: Matrix controlled transdermal patch system[8]

Adhesive Dispersion-Type Systems

This system is a simplified form of the membrane permeation-controlled system. Here the drug reservoir is formulated by directly dispersing the drug in an adhesive polymer e.g., poly (isobutylene) or poly (acrylate) adhesive and then spreading the medicated adhesive, by solvent casting or hot melt, on to a flat sheet of drug impermeable metallic plastic backing to form a thin drug reservoir layer. On the top of the drug reservoir layer, thin layers of non-medicated, rate controlling adhesive polymer of a specific permeability and constant thickness are applied to produce an adhesive diffusion-controlled delivery system.

For example, Isosorbide dinitrate releasing transdermal therapeutic system (Frandol tape/Yamanouchi, Japan) once-a-day medication of angina pectoris.

Micro reservoir Type or Micro sealed Dissolution Controlled Systems

This system is a combination of the reservoir and matrix diffusion type drug delivery systems. The drug reservoir is formed by first suspending the drug solids in an aqueous solution of a water-soluble liquid polymer and then dispersing the drug suspension homogenously in a lipophilic polymer viz. silicone elastomers by high-energy dispersion technique to form several discrete, unleachable microscopic spheres of drug reservoirs.

The quick stabilization of this thermodynamically unstable dispersion is accomplished by immediately cross-linking the polymer chains in-situ, which produces a medicated polymer disc with a constant surface area and fixed thickness. Positioning the medicated disc at the center and surrounding it with an adhesive produce a transdermal therapeutic system.

Nitroglycerin releasing transdermal therapeutic system (Nitro disc, Searle, USA) for once a day therapy of angina pectoris is a good example of this class.

REVIEW OF LITERATURE

📖 Dandagi PM *et al.*[18] prepared transdermal films of ketotifen fumarate using Eudragit L 100, Hydroxypropyl methyl cellulose and ethyl cellulose in combinations. Polyethylene glycol 400 was used as plasticizer. Permeation enhancers like dimethyl sulfoxide and propylene glycol were used at different concentrations and *in vitro* drug release studies were carried out. They concluded that as the concentration of permeation enhancers increased, the drug release increased.

📖 Mirohal A *et al.*[22] prepared transdermal formulations of testosterone designed for biphasic delivery, containing a blend of polymeric components polyvinyl alcohol and polyvinyl pyrrolidone in isopropyl alcohol. They reported that the film initially showed burst effect and then sustained release of testosterone.

📖 Kulkarni RV *et al.*[23] prepared and evaluated Eudragit RS 100 films as rate controlling membrane for transdermal use and studied the effect of various plasticizers on the permeability and mechanical properties using verapamil hydrochloride as a model drug. They reported that films plasticized with polyethylene glycol showed higher permeability of verapamil hydrochloride as compared to Dibutyl phthalate. They also reported that permeability of drug decreased as

the concentration of dibutyl phthalate and polyethylene glycol increased.

📖 Panigrahi L *et al.*[24] prepared pseudo-latex transdermal drug delivery system of terbutaline sulphate using combination of Eudragit RS 100 and eudraflex. They reported that the drug release profiles from the patches followed apparent zero order patterns for a period of 12 h and skin permeation took place at a steady rate over a period of 12 h after which the rate was reduced.

📖 Nagarajan M *et al.*[25] prepared gelatin films of salbutamol sulphate transdermal drug delivery system using ethyl cellulose film as rate controlling membrane. They reported that controlled release of salbutamol sulphate was observed for a period of 12-24 h and the drug release was found to depend on the proportion of gelatin used in drug reservoir layer. They showed that drug release from gelatin films can be controlled using ethyl cellulose film as rate controlling membrane.

📖 Sankar V *et al.*[26] prepared drug free polymeric films of ethyl cellulose to explore their suitability for transdermal application as rate controlling membrane using castor oil and glycerol as plasticizers. The permeability of free films were studied using nifedipine as model drug and reported that faster release was observed from ethyl cellulose patches containing glycerol as plasticizer.

📖 Narasimha MS *et al.*[27] carried out drug release studies from transdermal films of terbutaline sulphate using polymers such as hydroxypropylmethylcellulose and sodium carboxy methyl cellulose and reported that the hydroxypropylmethylcellulose films showed a greater rate of release compared to that of sodium carboxy methyl cellulose across all the barriers used.

📖 Mishra A *et al.*[28] prepared transdermal formulations of testosterone designed for biphasic delivery, containing a blend of polymeric components polyvinyl alcohol and polyvinyl pyrrolidone in isopropyl alcohol. They reported that the film initially showed burst effect and then sustained release of testosterone.

📖 Aqil M *et al.*[29] prepared and evaluated of monolithic matrix type transdermal drug delivery system of metoprolol tartarate using different concentration ratios of eudragit RL100 and polyvinyl pyrrolidone K-30. They reported that, the release of drug from the matrix was controlled and the skin permeation rate followed zero order kinetics.

📖 Gupta SP *et al.*[30] prepared metoprolol tartarate transdermal drug delivery system for controlled release of drug for extended period of time using Eudragit RL and Hydroxypropyl methyl cellulose. They reported that, the transdermal drug delivery system exhibited better and

constant drug plasma profile for 24 h as compared to oral administration.

📖 Jain GK *et al.*[31] studied the effect of penetration enhancer (α-limonene) on poly vinyl alcohol and polyvinyl pyrrolidone films containing verapamil hydrochloride as drug. They reported that the drug permeation was enhanced by using α- limonene as permeation enhancer.

📖 Narasimha MS *et al.*[32] prepared terbutaline sulphate transdermal films using various cellulose like sodium carboxy methyl cellulose, cellulose acetate, and ethyl cellulose. They studied the drug release from human cadaver skin and reported that, the films made from hydrophilic polymers showed a greater rate of release than that of hydrophobic polymers.

📖 Das MK *et al.*[33] studied the effect of polymeric composition, drug content and plasticizer on the permeation of trazodone hydrochloride across the mouse epidermis for the development of transdermal therapeutic system and carried out *in vitro* drug release studies. They observed that, drug release increased with increase in amount of Eudragit RL100 in the film. They demonstrated the potential use of fabricated pseudolatex transdermal films for sustained release of trazodone hydrochloride.

📖 Jin K *et al.*[34] carried out the feasibility studies of using EVA for developing transdermal delivery of atenolol. They studied the effect of drug concentration, temperature, and plasticizers on drug release from the atenolol-EVA matrix. They reported that the drug release from the matrix increased with increase in temperature and drug loading.

📖 Xiaoping Z *et al.*[35] studied new type of copolymer membranes through photosynthesis of mixtures of three different monomers 2-hydroxy-3-phenoxy Propyl acrylate, 4-hydroxy butyl acrylate and sec-butyl tiglate by using clonidine as model drug. They reported that the prepared membranes showed near zero order permeation rates.

📖 Michael HQ *et al.*[36] studied the release of permeation enhancers from transdermal drug delivery system of drug-in-adhesive type using known enhancers from eight types of adhesive polymers. They showed that, enhancers released completely from the adhesive and the release rate depended on the types of adhesives used especially among the acrylic polymers. They also showed that acrylic adhesive and polyisobutylene adhesive showed slower drug release rate than silicon adhesive.

📖 Kulkarni RV *et al.*[37] prepared drug free polymeric films of polyvinylpyrolidone (PVP), ethyl cellulose (EC), Eudragit RS 100 (Ed), and ethylene vinyl acetate (EVA) using verapamil HCl as model drug. The prepared films were evaluated for drug release study and

they showed that, drug diffusion followed nearly zero order kinetics and it is in the order of PVP > EC > Ed > EVA.

📖 Arora P *et al.*[38] prepared matrix type transdermal patches containing diclofenac diethylamine using different ratios of PVP and ethyl cellulose by solvent evaporation technique. The drug matrix films were casted on a polyvinyl alcohol backing membrane and physical studies (moisture content, moisture uptake, and flatness), *in vitro* release studies were studied. They concluded that diclofenac diethylamine can be formulated into the transdermal matrix type patches for sustaining its release characteristics and the polymeric composition (PVP/EC, 1:2) was found to be the best among the formulations studied.

📖 Kusum D *et al.*[39] developed and evaluated free films and transdermal patches of ketorolac tromethamine using polymers and pressure sensitive adhesives. PVP and PVA were used as polymer matrix materials and acrylic and silicone based pressure sensitive adhesives were used as adhesive matrix materials. They observed that the permeation of drug with Span 80 was more when compared to Tween 80, oleic acid, propylene glycol as enhancers.

📖 Ramesh *et al.*[40] developed transdermal reservoir patch of naloxone and evaluated for *in vivo* studies, stability studies, and irritancy potential. Propylene glycol and oleic acid have been used as

penetration enhancers and showed developed transdermal system of naloxone is efficacious, stable and safe upon single and multiple dose application.

Table 02: Commercially available TDDS[41,42]

Therapeutic agent	Trade name	Manufacturer	Strengths available
Clonidine	Catapress-TTS	Boeh-ringer Inyelheim	2.5 mg, 5.0 mg, 7.5 mg
Estradiol	Estraderm	Ciba	25 µg, 50 µg, 100 µg
Fentanyl	Duragesic	Janssen	25 µg, 50 µg, 75 µg, 100 µg
Nicotine	Habitrol	Basel	21 µg
	Nicoderm	Marion merrell	21 µg
	Nicotrol	Parke-davis	15 µg
	Prostep	Lederle	22 µg
Nitroglycerin	Deponit	Schwarz Pharm	0.4 mg, 0.2 mg
	Nitrodisc	Searle	0.3 mg, 0.2 mg
	Nitro-dur II	Key	0.1 mg, 0.2 mg, 0.6 mg
	Transderm nitro	Ciba	0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.6 mg
Scopolamine	Transderm Scop	Ciba	1.5 mg

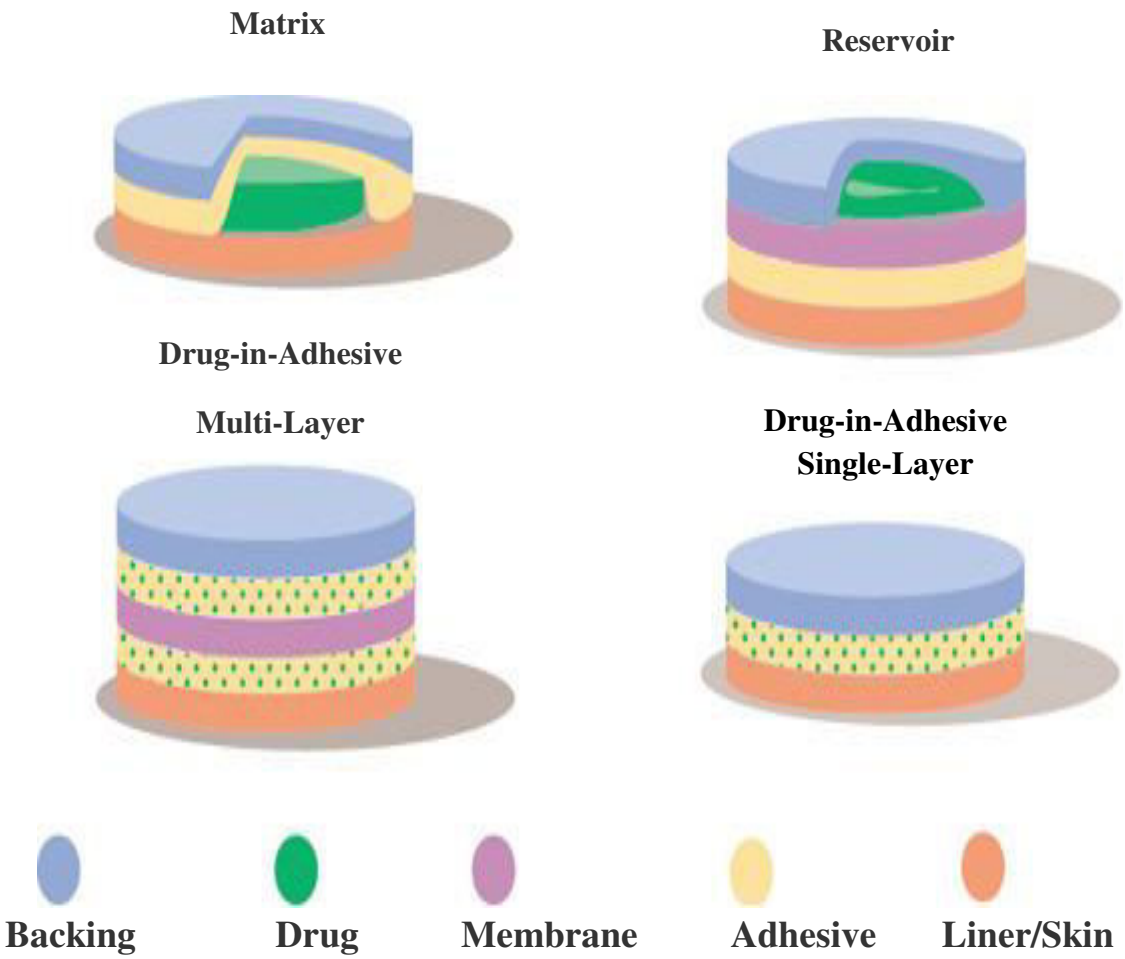


Figure 5: Different transdermal drug delivery systems

Keeping in view of the scientific reports enumerated in this chapter, suitable experimental methodology was adopted to obtain the answers for the questions given in the chapter "objectives".

OBJECTIVES

The absorption of drugs through the transdermal route improves bioavailability of drugs that might otherwise be metabolized by first-pass effect (pre-systemic drug elimination) during their passage through the gastrointestinal tract. Drug absorption from the transdermal route is mainly via passive diffusion through the lipoidal membrane. Thus, transdermal route of drug delivery has attracted the attention worldwide for optimizing the drug delivery.[3]

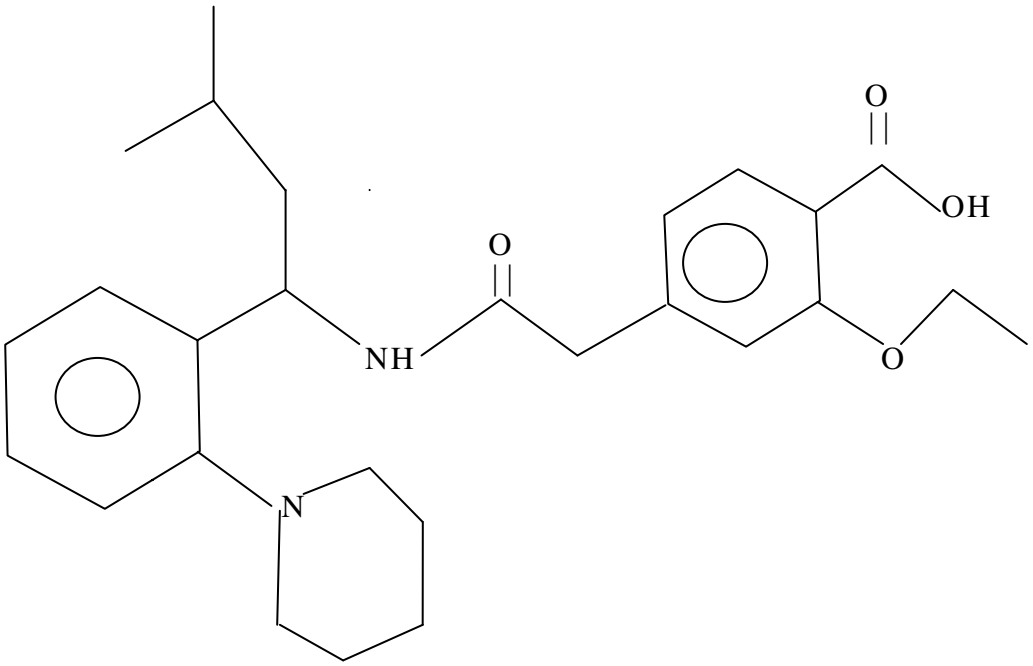
The objectives of the present investigation are**:**

1. To design suitable transdermal films of Repaglinide using bioadhesive polymers.
2. To characterize developed films for their *in vitro* drug release / permeation studies.
3. To formulate and evaluate the transdermal patches / films for the following parameters;
   - ❖ Thickness uniformity.
   - ❖ Weight uniformity.
   - ❖ Swelling index.
   - ❖ Tensile strength.
   - ❖ Folding endurance.
   - ❖ Water vapour transmission rate.
   - ❖ Drug content uniformity.
   - ❖ *In vitro* drug release studies.
4. To carry out short term stability studies on the most satisfactory formulation as per ICH guidelines.

DRUG PROFILE

REPAGLINIDE [9,10,11,12]

Structure: It is a racemic mixture with the following structure:



Molecular Formula : $C_{27}H_{36}N_2O_4$.

Molecular Weight : 452.5857

IUPACName: 2-ethoxy-4-[{[(15}-3-methyl-1-[2-(piperdinyl)phenyl]butylcarbamoyl}methyl]benzoli cacid

Description: White to off-white crystalline powder; hygroscopic.

Solubility : It is freely soluble in methanol and acetone; sparingly soluble in 95% ethanol and isopropanol; partially soluble in water.

Category : Repaglinide is a non sulphonyl urea oral hypoglycaemic agent

Use : Oral hypoglycaemic agent

Melting Point : Melting point is in between 129 to 130 ^{o}C.

Mechanism of action: Repaglinide is a new class of non sulphonyl urea oral hypoglycaemic agent. It reduces the blood glucose by stimulating the release of insulin from the pancreas, distinct from all other antidiabetic agent in their chemical structure, mechanism of binding to target channels in beta-cells, and mode of elimination. These agents have several desirable properties including a rapid onset and short duration of action and metabolism, and excretion by non-renal routes. The bioavailability is approximately 45% and half life is 1 - 3 hours.

Pharmacokinetics: Repaglinide is well absorbed from gastrointestinal tract but it subjected to high first pass metabolism in liver; the absolute bioavailability is about 56% Peak plasma concentration occurs in 1-3 hours after drug administration. It has high lipid solubility. Repaglinide is effective in lowering fasting blood glucose concentrations (-13%), glycosolated haemoglobin(0.6-1.0%), 2 hour postprandial plasma glucose concentration-time profile.

Therapeutic Uses: It has antidiabetic activity on type two diabetes mainly which occurs in youths and in old aged people and with combination with drugs of antidiabetic class of type one is found to be more effective than the plain drugs used in treatment of type one diabetes. at higher doses calcium channel blocking activity may contribute.

Adverse Effects: Acute renal failure and renal abnormalities have been reported in patients with heart failure who also suffered from diffuse vascular disease and/or renal impairment.

Contraindications:

Therapy with repaglinide is contraindicated in patients with

1. Congestive heart failure requiring pharmacological treatment
2. Renal disease or renal dysfunction, which may also result from conditions such as cardiovascular collapse, acute myocardial infarction and septicaemia.
3. Known hypersensitivity to repaglinide.
4. Acute or chronic metabolic acidosis, including diabetic ketoacidosis with or without coma. Diabetic ketoacidosis should be treated with insulin.

Storage: Protect from moisture, Stored in tight light-resistant container.

Dose: 2, 4, 10, 20 and 25.0 mg.

POLYMER REVIEW

1)Hydroxy Propyl Methyl Cellulose: [13,14,15]

Non Proprietary Name :

British pharmacopoeia: Hypromellose

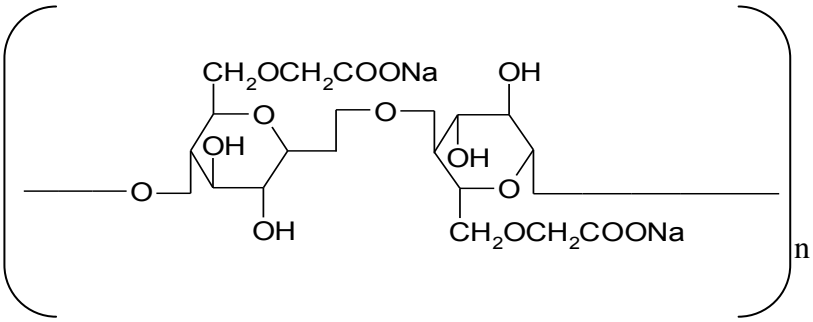
United State Pharmacopoeia: Hydroxy Propyl Methyl cellulose

Synonyms : Methocel, HPMC

Chemical Name : Cellulose, 2-Hydroxypropyl methyl ether

Empirical Formula : HPMC is a partially o-methylated and o-(2-Hydroxy propylated)

Structural Formula :



Molecular Weight : Approximate 10000 to 1500000.

Functional Category : Tablet binder, coating agent, and film former.

Pharmacopoeia : BP and USP.

Description : Odourless, tasteless, white or creamy white fibrous or granular powder.

pH : 5.5 to 8.0.

Aqueous Viscosity : (1% w/v) HPMC K4M = 3000 to 5600 cp.
HPMC K15M = 11250 to 21000 cp.

Solubility : Soluble in cold water, insoluble in alcohol, ether, and chloroform, but soluble in a mixture of methylene chloride and methanol.

Stability : Stable in dry condition from pH 3.0 to 11.0.

Storage Condition : It is hygroscopic in nature. Should be stored in well-closed container, in a cool and dry place.

Incompatibilities : Incompatible with some oxidizing agents. Since it's non-ionic, hydroxypropylmehyl cellulose will not complex with metallic effect.

Safety : It's generally regarded as a nontoxic and non-irritant material although excessive oral consumption may have a laxative effect.

Application : HPMC is widely used in oral and topical pharmaceutical formulations. In oral products, it primarily used tablet binder and extended release matrix.

ETHYLCELLULOSE[16]

Synonym : Ethylcellulosum

Functional category: Coating agent, tablet binder, viscosity-increasing agent. **Description:** Ethyl cellulose is a tasteless, free-flowing, white to light tan coloured powder.

Solubility: Ethyl cellulose practically insoluble in glycerine, propylene glycol and water. Ethyl cellulose is soluble in chloroform, methyl acetate, tetrahydrofuran, and in mixture of aromatic hydrocarbons with ethanol (95%).

Stability and storage conditions: Ethyl cellulose is a stable, slightly, hygroscopic materials. It is chemically stable to alkalis, both dilute and concentrated, and to salt solutions, although it is more sensitive to acidic materials than cellulose esters. The bulk materials should be

stored in a dry place, in a well closed container at a temperature between 7-32 ºC.

Incompatibilities: Incompatible with paraffin wax and microcrystalline wax.

Application: Ethyl cellulose is widely used in oral and topical pharmaceutical formulations. The main use of ethyl cellulose in oral formulations is as a hydrophobic coating agent for tablets and granules. Ethyl cellulose coatings are used to modify the release of drug. Ethyl cellulose, dissolved in an organic solvent or solvent mixture, can be used on its own to produce water-insoluble films. Higher viscosity ethyl cellulose grades are used to produce stronger and tougher films. In topical formulations, ethyl cellulose is used as a thickening agent in creams, lotions or gels, provided an appropriate solvent is used. Ethyl cellulose is additionally used in cosmetics and food products.

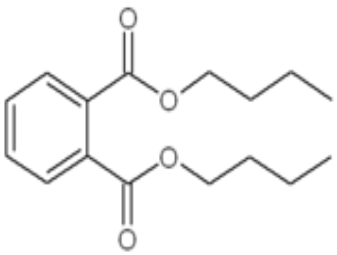
PLASTICIZER REVIEW

1) DIBUTYL PHTHALATE [17]

Synonym : Butyl phthalate; DBP

Chemical Name : Dibutyl benzene,1,2-dicarboxylate

Structural Formula:



Molecular Formula : $C_{16}H_{22}O_4$

Molecular Weight	**:**	278.3
Description	**:**	A clear, colourless or faintly yellow, somewhat viscous.
Density	**:**	1.045
Boiling Point	**:**	330 ^{o}C
Refractive Index	**:**	1.492 to 1.495
Solubility	**:**	Very soluble in ethanol, ether, acetone, benzene, 1 in 2500 parts of water, miscible with ethanol, ether and most other organic solvents.

PERMEATION ENHENCHER REVIEW

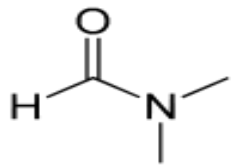
***1)* DIMETHYL FORMAMIDE:**[17]

Synonyms: DMF,Dimethylformamide, N,N-dimethylformamide, DMFA

Description: Colourless liquid, miscible with water and the majority of organic liquids.

Chemical Name: N,N-dimethylmethanamide

Structural Formula:



Molecular Formula **:** C_3H_7NO

Molecular Weight **:** 73.09 g/mol

Density **:** 0.944 g/cm^3

Boiling Point **:** 153 °C

Refractive Index **:** 1.4305 (20 °C)

Viscosity: 0.92 CP at 20 °C

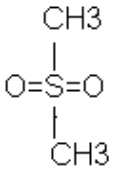
Solubility : Miscible with water, alcohol, ether; acetone, benzene.

Storage Condition : Stored in air tight glass containers, protected from light.

(2) DIMETHYL SULFOXIDE: [18]

Synonyms : Dimethyl sulfoxide, DMSO, Methyl sulfoxide

Description : A colourless hygroscopic odourless liquid or crystals.



Structural Formula:

Molecular Formula : C_2H_6 OS

Specific Gravity : 1.100 to 1.104

Molecular Weight : 78.13

Solubility : Miscible with water, alcohol, ether, acetone, benzene.

Storage condition : Store in air tight glass containers, protect from light.

Application : DMSO is highly polar substance which has exceptional solvent properties for both organic and inorganic chemicals and is widely used as an industrial solvent. The principal use of DMSO is as a vehicle for drug, it aids penetration of drug into the skin, mucus layer and so may enhance the drug's bioavailability.

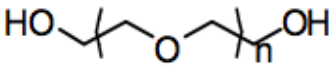
SURFACTANT REVIEW

1) TWEEN 80[19]

Synonyms	**:**	Polyoxyethylene 20 oleate, Tween 80, Polysorbate 80.
Chemical Name	**:**	Polyoxyethylene 20 sorbitan mono oleate.
Empirical Formula	**:**	$C_{64}\ H_{124}\ O_{26}$
Molecular Weight	**:**	1310.
Functional Category	**:**	*Emulsifying agent; non-ionic surfactant; solubilizing agent, wetting agent.*
Description	**:**	*Polysorbates have a characteristic odour and somewhat bitter in taste. Their colour and physical form at 25 ^{o}C is yellow oily liquid.*

2) POLYETHYLENE GLYCOL-400[21]

Synonyms: poly (ethylene oxide) (PEO) or polyoxyethylene (POE), polyether's

Structural Formula: 

Description	**:**	A clear, colourless, viscous and practically odourless liquid having a sweet, slightly acrid taste resembling glycerol.
Molecular Formula	**:**	$C_{2n}H_{4n+2}O_{n+1}$
Molecular Weight	**:**	380-420 g / mol
Density	**:**	1.128 g / cm^3

Boiling Point	**:**	182 - 287 °C
Viscosity	**:**	90.0 cSt at 25 °C, 7.3 cSt at 99 °C
Solubility	**:**	PEG is soluble in water, methanol, benzene, dichloromethane and insoluble in diethyl ether and hexane.
Storage Condition	**:**	Store in air tight glass containers, protect from light.

MATERIALS AND METHODS

Distilled water was prepared in the laboratory using all glass distillation apparatus.

Experimental Methods

Analytical methods of Repaglinide

- Determination of λ_{max} of repaglinide in 0.1 N HCl solution
- Determination of λ_{max} of repaglinide in pH 7.4 phosphate buffer solution
- Calibration curve of repaglinide 0.1N HCl solution
- Calibration curve of repaglinide in pH 7.4 phosphate buffer solution

Preformulation Studies

- Determination of melting point
- Determination of partition coefficient
- Determination of drug-excipients compatibility studies

Evaluation of film / patch formulation

- Thickness uniformity
- Weight uniformity
- Tensile strength
- Swelling index
- Folding endurance
- Water vapour transmission rate
- Drug content uniformity of films
- *In vitro* drug release studies
- Stability studies

Preparation of Solutions[42]

Phosphate buffer (pH 7.4) solution: Fifty ml of 0.2M potassium dihydrogen phosphate was taken in 200 ml volumetric flask, to which 39.1 ml of 0.2 M sodium hydroxide solution was added and the volume was made up to the mark with distilled water.

Potassium dihydrogen phosphate (0.2 M) solution: Potassium dihydrogen phosphate (27.218) g was added to 1000 ml volumetric flask containing distilled water and the volume was made up to the mark with distilled water.

Sodium hydroxide (0.2 M) solution: Eight gram of sodium hydroxide was taken in 1000 ml volumetric flask containing distilled water and volume was made up to the mark with distilled water.

Analytical Methods

Determination of λ_{max} of repaglinide in Phosphate Buffer (pH 7.4) Solution :

Preparation of Repaglinide standard stock solution (200 µg/ml) in methanol: Standard stock solution of repaglinide was prepared by dissolving accurately weighed 10 mg of repaglinide in the little quantity of methanol in 50 ml volumetric flask. The volume was then made up to 50 ml by using methanol to obtain the solution of 200 µg/ml.

Scanning of repaglinide by UV-spectrophotometer in 0.1 N HCl solution: From the standard stock solution, 1 ml was pipetted into a volumetric flask. The volume was then made up to 20 ml with 0.1 N

HCl solutions. The resulting solution containing 10 µg/ml was scanned between 200 - 400 nm. The λ_{max} was found to be 237 nm.

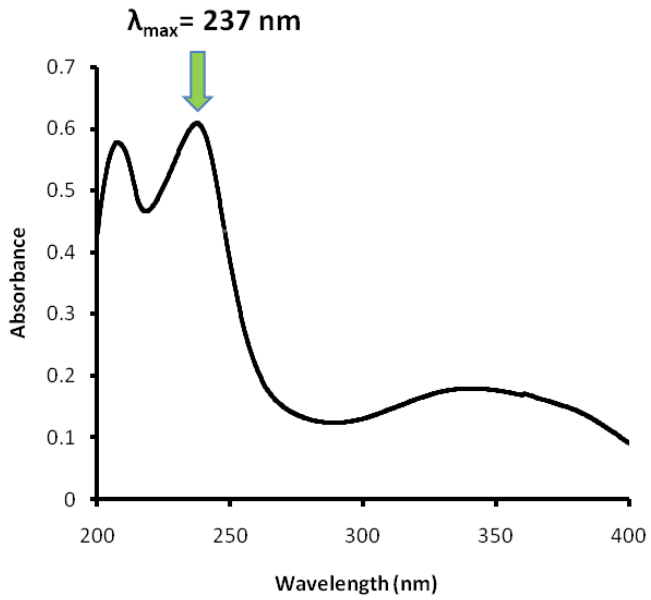


Figure 6: UV spectrum of Repaglinide in 0.1 N HCl solutions (10 µg/ml).

Calibration curve of Repaglinide in 0.1 N HCl solution: From the repaglinide standard stock solution (200 µg/ml), 1 ml was pipetted and diluted to 20 ml by using 0.1 N HCl solutions. From this solution, appropriate aliquots were taken into different volumetric flasks and made up to 10 ml with 0.1 N HCl solutions, so as to get drug concentrations from 2.5µg/ml to 20.0 µg/ml. The absorbencies of these drug solutions were estimated at λ_{max} 237 nm. This procedure was performed in triplicate to validate the calibration curve. The data are given in Table 03. A calibration curve was constructed as shown in Figure 07. Calibration equation and regression value are shown in Figure 07.

Table 3: **Data for calibration curve of repaglinide 0.1 N HCl solution at 237 nm**

Sl. No.	**Concentration, µg/ ml**	*** Absorbance at 237 nm AM ± SD**
1	0	0.000 ± 0.000
2	2.5	0.115 ± 0.002
3	5.0	0.216 ± 0.005
4	7.5	0.345 ± 0.007
5	10.0	0.483 ± 0.006
6	12.5	0.552 ± 0.007
7	15.0	0.675 ± 0.007
8	17.5	0.798 ± 0.002
9	20.0	0.903 ± 0.004

* Each value was an average of three determinations

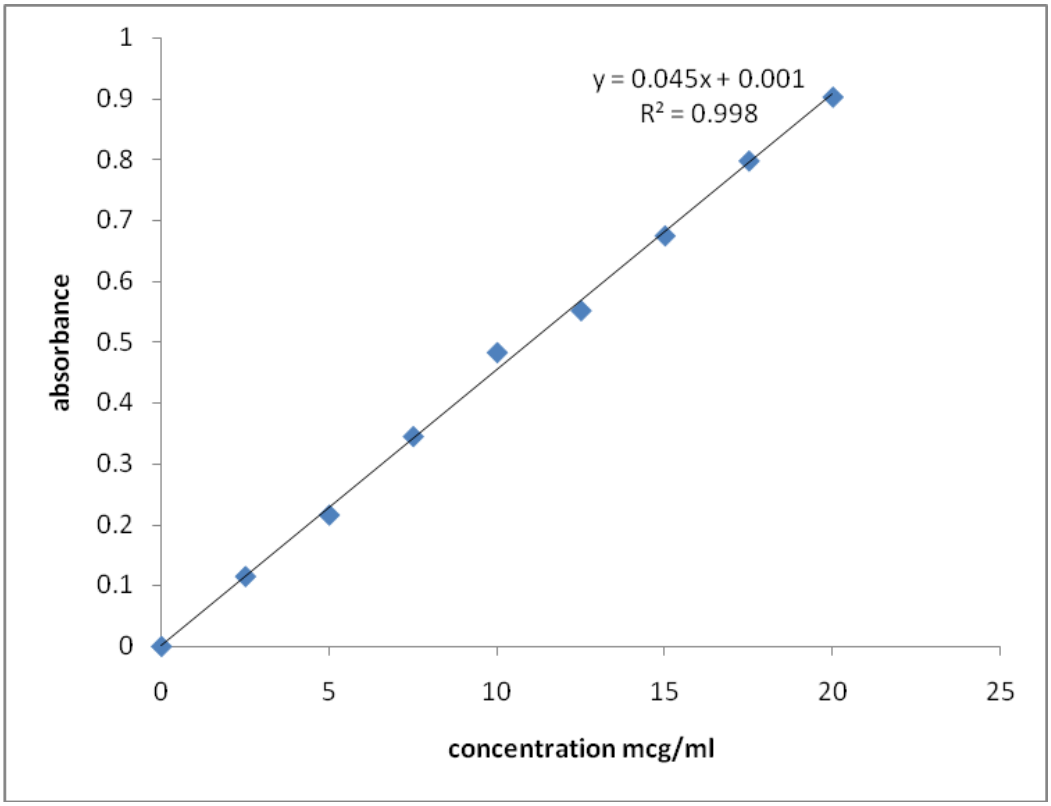


Figure 7: Standard plot of repaglinide in 0.1 N hydrochloric acid solutions.

Scanning of Repaglinide by UV-spectrophotometer in phosphate buffer (pH 7.4) solution: From the standard stock solution, 1 ml was diluted to 20 ml with phosphate buffer solution (pH 7.4). The resulting solution containing 10 µg/ml was scanned between 200 and 400 nm. The λ_{max} was found to be 237 nm.

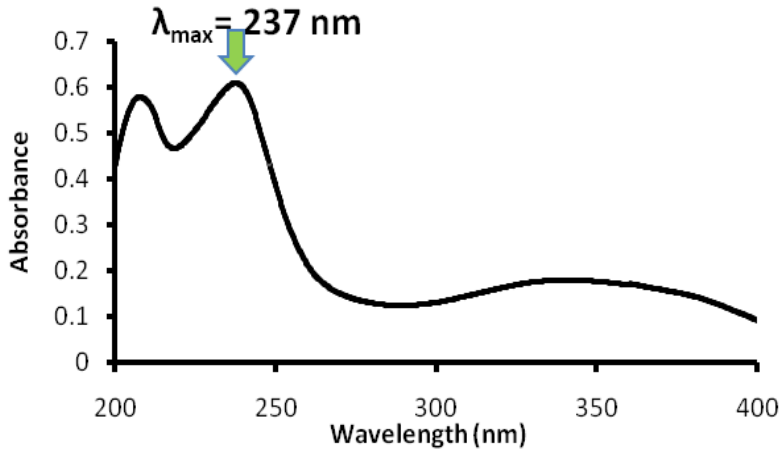


Figure 8: UV spectrum of Repaglinide in 7.4 pH phosphate buffer solution (10 µg/ml)

Calibration curve of Repaglinide in phosphate buffer solution (pH 7.4): From the repaglinide standard stock solution (200 µg/ml), 1 ml solution was diluted to 19 ml using 7.4 pH phosphate buffer solutions. Appropriate aliquots were taken into different volumetric flasks and made up to 10 ml with phosphate buffer solution (pH 7.4), so as to get drug concentrations of 2.5 µg/ml to 22.5 µg/ml. The absorbencies of these drug solutions were estimated at λ_{max} 237 nm. This procedure was performed in triplicate to validate the calibration curve. The data are given in Table 04. A calibration curve was constructed as shown in Figure 09. Calibration equation and regression value are shown in Figure 09.

Table 4: Data for calibration curve of repaglinide phosphate buffer solution (pH 7.4) at 237nm

Sl. No.	Concentration, µg/ ml	* Absorbance at 237 nm
1	0	0.000 ± 0.000
2	2.5	0.117 ± 0.002
3	5.0	0.218 ± 0.004
4	7.5	0.347 ± 0.008
5	10.0	0.487 ± 0.007
6	12.5	0.557 ± 0.004
7	15.0	0.675 ± 0.003
8	17.5	0.798 ± 0.004
9	20.0	0.907 ± 0.005
10	22.5	1.105 ± 0.006

* Each value was an average of three determinations

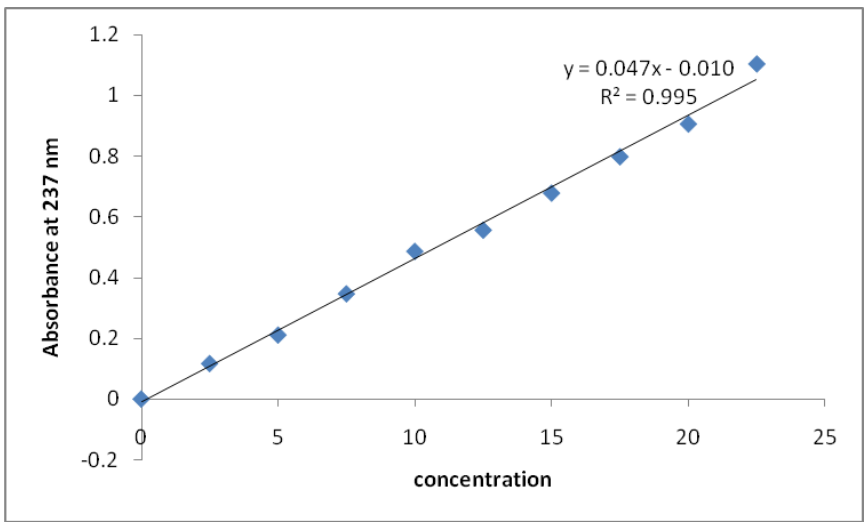


Figure 9: Standard plot of repaglinide in phosphate buffer solution (pH 7.4)

Interference of Polymers in the Estimations

Scanning of HPMC solutions (0.2%) in phosphate buffer solution (7.4 pH): It was necessary to identify the incompatibility of polymer and drug for the analysis. Keeping in view of the concentration of polymer, an empirical concentration was fixed for the study of analysis. Solutions of polymers were prepared as per the concentrations given in the Table 05. The solutions were scanned in UV- region, 200-400 nm, using corresponding blank solutions. Figures 10- 12 represent the UV scans of polymeric solutions.

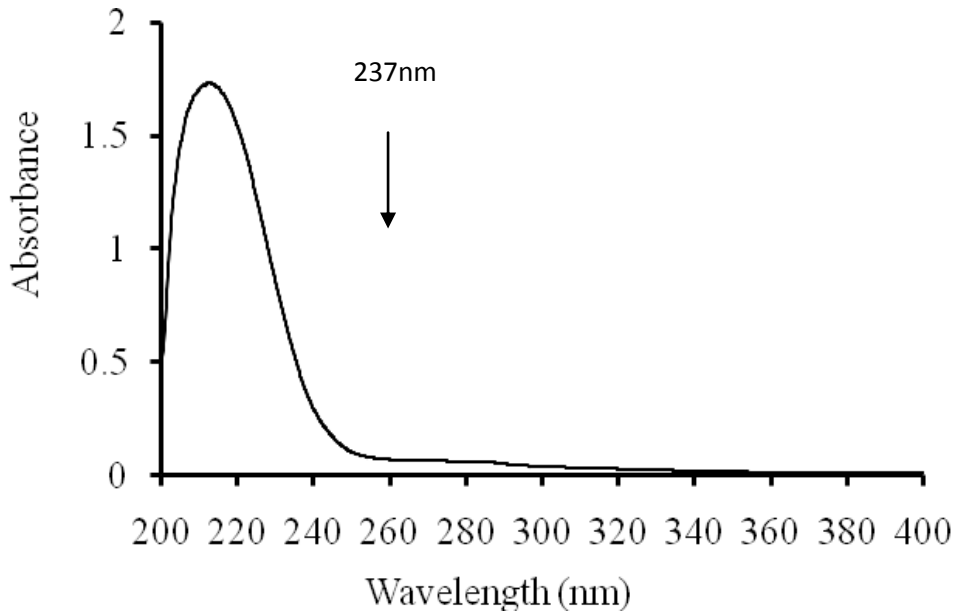


Figure 10:UV- spectrum of HPMC in 7.4 pH phosphate buffer solution (0.2 % w/v)

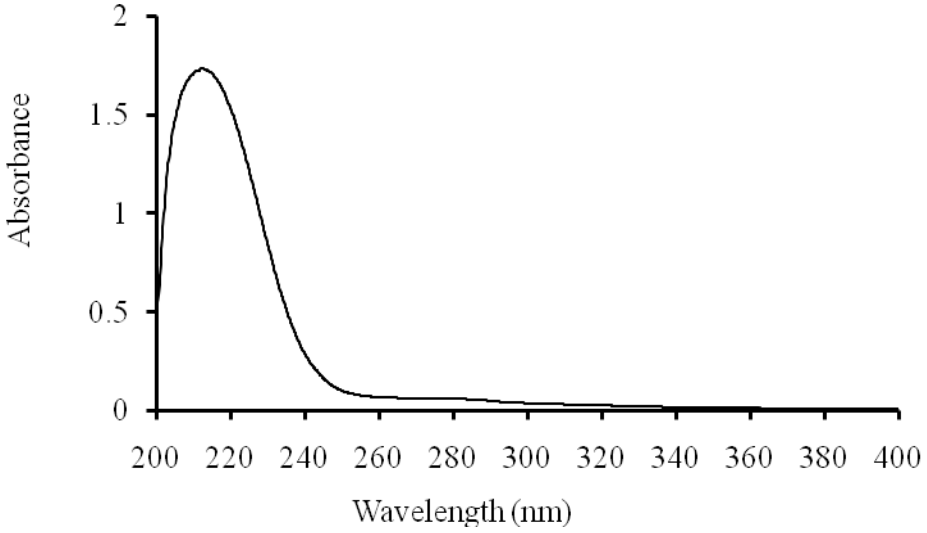


Figure 11: UV- spectrum of Ethyl cellulose (0.1% w/v) in ethanol.

Table 5: **Data for estimation of interference of polymers**

Polymer	Concentration % w/v	Solvent	Absorbance at 241 nm
HPMC K 100	0.2	Buffer	0.1043
Ethyle cellulose	0.2	Methanol	0.1063

At the wavelength of 237 nm, the polymer solutions had not shown appreciable absorbance. In other words; polymer does not interfere with the absorption of drug.

Fourier Transform Infrared Spectroscopy

The pure drug (Repaglinide) and polymers were subjected to IR studies alone and in combination. Pure drug/pure polymer/combination of drug-polymer were mixed with 100 mg of potassium bromide. Thorough grinding in smooth mortar can effect mixing. The mixtures

were then placed in the sample holder of the instrument. These were analyzed by FTIR to study the interference of polymer for drug analysis.

Preparation of Transdermal Films / Patches of Repaglinide

Transdermal films containing repaglinide were prepared by the solvent evaporation technique for the formulations shown in Table 06. Solution of HPMC K-100 and Ethyl cellulose were prepared separately in ethanol. The two polymeric solutions were mixed to which weighed amount of repaglinide was added slowly. To the mixture, 4 drop dibutyl phthalate (0.15 mg). The drug-polymer solution was casted in a glass mould of 15 cm^2 (3 × 5 cm^2). The mould was kept aside for drying at room temperature for 24 h. Inverted plastic funnel was placed over the mould to prevent the current of air. After drying the films were peeled from glass mould, wrapped in aluminium foil, and preserved in desiccator for further studies. In the literature, the terms "patches" and "films" were used synonymously. In this thesis, the two terms are carefully used to avoid confusion. The term "patch" refers to 1 x 1 cm^2 size formulations and the term "film" refers to the formulation of bigger size than patch.

Table 6: Composition of different formulations containing Repaglinide

INGEDIENTS	F1	F2	F3	F4	F5
Repaglinide	10mg	10mg	10mg	10mg	10mg
Ethyl cellulose	400mg	300mg	200mg	100mg	0mg
HPMC K-100	0mg	100mg	200mg	300mg	400mg
DBP	0.015mg	0.015mg	0.015mg	0.015mg	0.015mg

Preformulation Studies

The following Preformulation studies were performed for Repaglinide and polymers;

1. Determination of melting point.
2. Determination of partition coefficient.
3. Determination of drug-excipients compatibility studies.

1. Determination of melting point

Melting point of the drug was determined by taking small amount of drug in a capillary tube closed at one end. The capillary tube was placed in a melting point apparatus and the temperature at which drug melts was recorded. This was performed thrice and average value was noted.

2. Determination of partition coefficient[43, 44]

The oil-water partition coefficient is a measure of lipophilicity of a molecule, which can be used to predict its capability to cross biological membrane. One of the most common ways of measuring partition coefficient is shake flask method.

Procedure: The Repaglinide was added little at once into 5 ml of *n*-octanol until saturated solution was obtained. This solution was filtered to get a clear solution. Three ml of the saturated solution was mixed with 2 ml of fresh *n*-octanol. In total 5 ml of *n*-octanol containing repaglinide was mixed with 15 ml of water. Then two phases were allowed to equilibrate at 37 ^{o}C for 24 h, on cryostatic constant temperature shaker bath (Research and Test Equipments, Bangalore, India). The concentration of the drug in the aqueous phase and organic phase was determined by *UV* spectroscopic method after necessary dilution. The apparent partition coefficient *(Kp)* was calculated as the ratio of drug concentration in each phase by the following equation.

$$K_P = \frac{C_{org}}{C_{aq}}$$

where, C_{org} is concentration of drug in organic phase and C_{aq} is the concentration of drug in aqueous phase.

3. Drug– excipients compatibility studies[33]

In the preparation of film formulation, drug and polymer may interact as they are in close contact with each other, which could lead to the instability of drug. preformulation studies regarding the drug-polymer interaction are therefore very critical in selecting appropriate polymers. FT-IR spectroscopy was employed to ascertain the compatibility between repaglinide and the selected polymers. The pure drug and drug with excipients were scanned separately.

Procedure: Potassium bromide was mixed with drug and/or polymer and the spectra were taken. FT-IR spectrum of repaglinide was compared with FT-IR spectra of repaglinide with polymer. Disappearance of repaglinide peaks or shifting of peak in any of the spectra was studied.

Thickness Uniformity of Films[22,23,24,26]

The thickness of each film was measured by using screw gauze. The thickness was measured at six different places on each film and the average thickness of the film was taken as the thickness of the film.

Weight Uniformity of Patches [22,23,24,26]

The patch of size 1x1 cm^2 was cut and weight of each patch was taken individually, the average weight of the patch was calculated.

Swelling Index[27]

Weight increase due to swelling and area increase due to swelling were studied.

a) **Weight increase due to swelling:** The drug-loaded patch of size 1 x 1 cm^2 was weighed on a pre-weighed cover slip. It was kept in a petridish and 50 ml of phosphate buffer (pH 7.4) solution was added. After every five min, the cover slip was removed, wiped with tissue paper, and weighed up to 30 min. The difference in the weights gives the weight increase due to absorption of water and swelling of patch.

b) **Area increase due to swelling*:*** The drug loaded patch of size 1 x 1 cm^2 was cut and placed in a petridish containing 50 ml of phosphate buffer (pH 7.4) solution. A graph paper was placed beneath the petridish and was clearly visible, which facilitated the measurement

of increase in the area. An increase in the length and breadth of the patch was noted at five min intervals for 60 min and the area was calculated. The percent swelling, % *S* was calculated using the following equation:

$$\%S = \frac{X_t - X_o}{X_o} \times 100$$

where X_t is the weight or area of the swollen patch after time *t* and X_o is the original patch weight or area at zero time.

Tensile Strength of Films [28, 29]

Tensile strength of the film was determined with Universal Strength Testing Machine (Hounsfield, Slinfold, Horsham, U.K.) as shown in Figure 13. The sensitivity of the machine was 1 gram. It consisted of two load cell grips. The lower one was fixed

Figure 12: Universal Strength Testing Machine

and upper one was movable. The test film of size (4 × 1 cm^2) was fixed between these cell grips and force was gradually applied till the film broke. The tensile strength of the film was taken directly from the dial reading in kg. Tensile strength is expressed as follows;

$$\text{Tensile strength} = \frac{\text{Tensile load at break}}{\text{Cross sectional area}}$$

Folding Endurance [30, 31]

The folding endurance was measured manually for the prepared films. A strip of film (4 x 3 cm) was cut and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the value of folding endurance.

Water Vapour Transmission (WVT) Rate [30,31]

For this study vials of equal diameter were used as transmission cells. These cells were washed thoroughly and dried in an oven, about 1 g of fused calcium chloride was taken in cells and the polymeric patches measuring 1 cm^2 area were fixed over the brim with the help of an adhesive. The cells were weighed accurately and initial weight was recorded, and then kept in a closed desiccator containing saturated solution of potassium chloride to maintain 63 % RH. The cells were taken out and weighed after 72 h.[14] The amount and rate of water vapour transmitted was calculated by the difference in weight using the formula:

$$\text{WVT rate} = WL/S$$

where, *W* is the water vapour transmitted in grams, *L* is the thickness of the patch in cm, and *S* is the exposed surface area in square cm.

Drug Content Uniformity[30,31]

The films were tested for the content uniformity. The film of size 2 × 2 cm^2 was cut and placed in a volumetric flask. Ten ml of methanol was added and the contents were stirred in a shaker bath for 24 h to dissolve the film. Subsequent dilutions were made with phosphate buffer (pH 7.4). The absorbance of the solution was measured against the corresponding blank solution at 241 nm using UV-VIS spectrophotometer. The experiment was repeated two more time to validate the result.

***In Vitro* Release Studies of Repaglinide from Transdermal Films**

***In vitro* release studies by using dissolution apparatus (Paddle over disk method):**[32,33] The drug release was determined using U.S.P. dissolution test apparatus (paddle over disk type method) thermo stated at 37±1 ^{o}C and stirred at a rate of 50 rpm. Sink condition was maintained throughout the study.

Each film was fixed on glass slide with the help of cyanoacrylate adhesive, so that the drug could be released only from upper face. The slide was immersed in the vessel containing 900 ml of phosphate buffer, pH 7.4, solution. Aliquots of 5 ml of sample were withdrawn with graduated pipette at every one hour time intervals up to 24 h replacing with equal volume of phosphate buffer.

The sample was analyzed spectrophotometrically at 237 nm and the cumulative amount of drug released at various time intervals was calculated. The test was carried out in triplicates.

Stability Studies[34]

Stability is defined as the ability of particular drug or dosage form in a specific container to remain within its physical, chemical, therapeutic, and toxicological specification. Drug decomposition or degradation occurs during stability, because of chemical alteration of the active ingredients or due to product instability, lowering the concentration of the drug in the dosage form. The stability of pharmaceutical preparation should be evaluated by accelerated stability studies. The optimized formulation of repaglinide films was selected for the stability studies. The accelerated stability studies were carried out according to ICH guidelines by storing the samples at 40 $\pm$ 2 ^{o}C and 75 $\pm$ 5% RH for 1 month. These samples were analyzed and checked for changes in physical appearance and drug content at an interval of 7 days.

RESULTS AND DISCUSSION

Preformulation Studies

The following Preformulation studies were performed for repaglinide.

Melting Point

Melting point of repaglinide was determined by capillary tube method and it was found to be 130° ± 1° (n = 3). This value is same as that of the literature citation.

Partition Coefficient

Partition coefficient determination study of repaglinide was done with n-octanol and water. The logarithmic value of partition coefficient (log pka) of repaglinide was found to be 3.497. This indicates that repaglinide is lipophillic in nature.

Table 7: **Data of various preformulation studies**

Sl. no.	Drug	Melting point	Partition coefficient
1.	Repaglinide	130 ± 1	5.90

*Standard deviation, n = 3

Drug – Excipients Compatibility Studies

As described in the methodology section the Fourier Transform infrared spectroscopy studies were carried out for pure drug along and along with polymers. The results are summarized as follows.

IR spectra of Repaglinide, HPMC K-100, Ethyl cellulose, alone and their combinations are shown in Figures 15 to 19. An IR spectrum of pure repaglinide shows the following peaks.

Table 8:FTIR spectrum of pure repaglinide

Bond (stretching)	**Wave number (cm^{-1})**
-NH	3344.93
-OH	2922.59
-CH	2630.43

The above peaks can be considered as characteristic peaks of repaglinide. These peaks were not affected and prominently observed in IR spectra of repaglinide along with polymers as shown in Figures 13 to 17. This indicates there is no interaction.

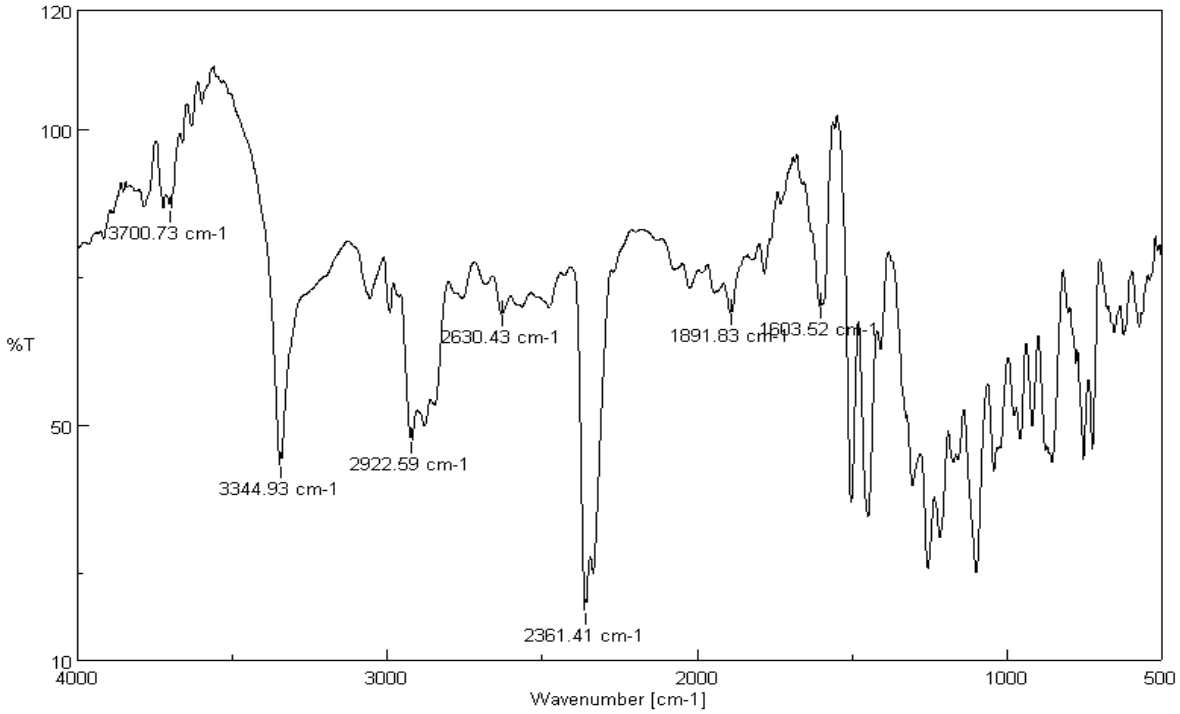


Figure 13: IR spectrum of repaglinide pure

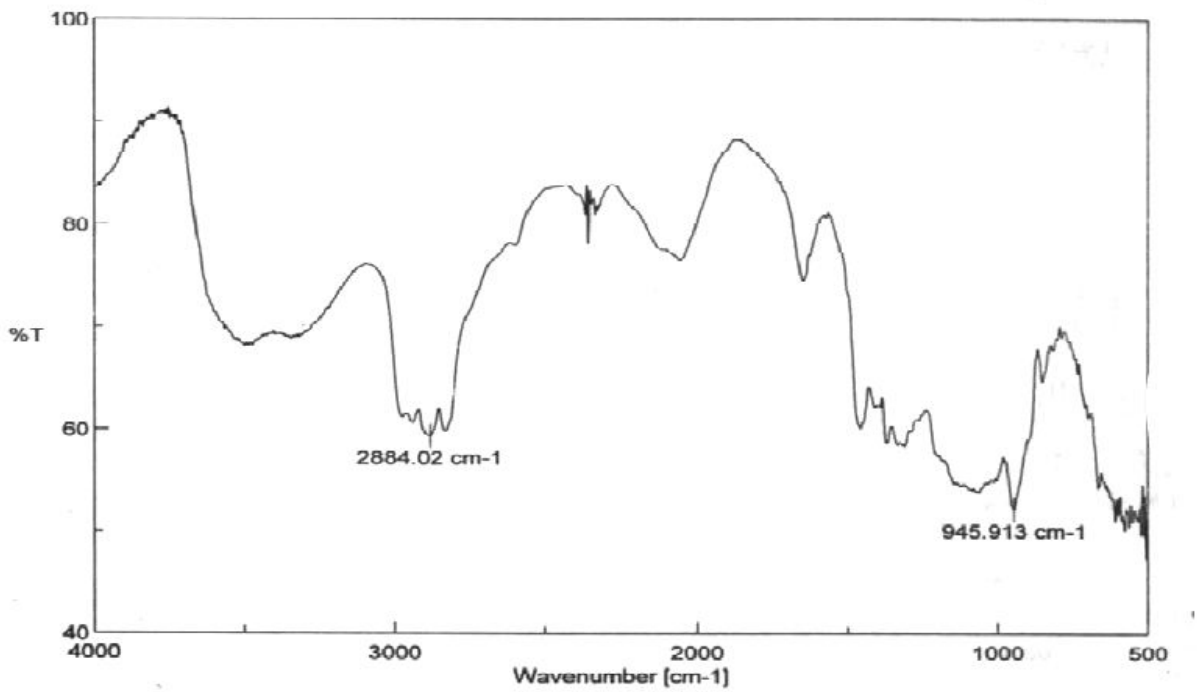


Figure 14: IR spectrum of HPMC pure

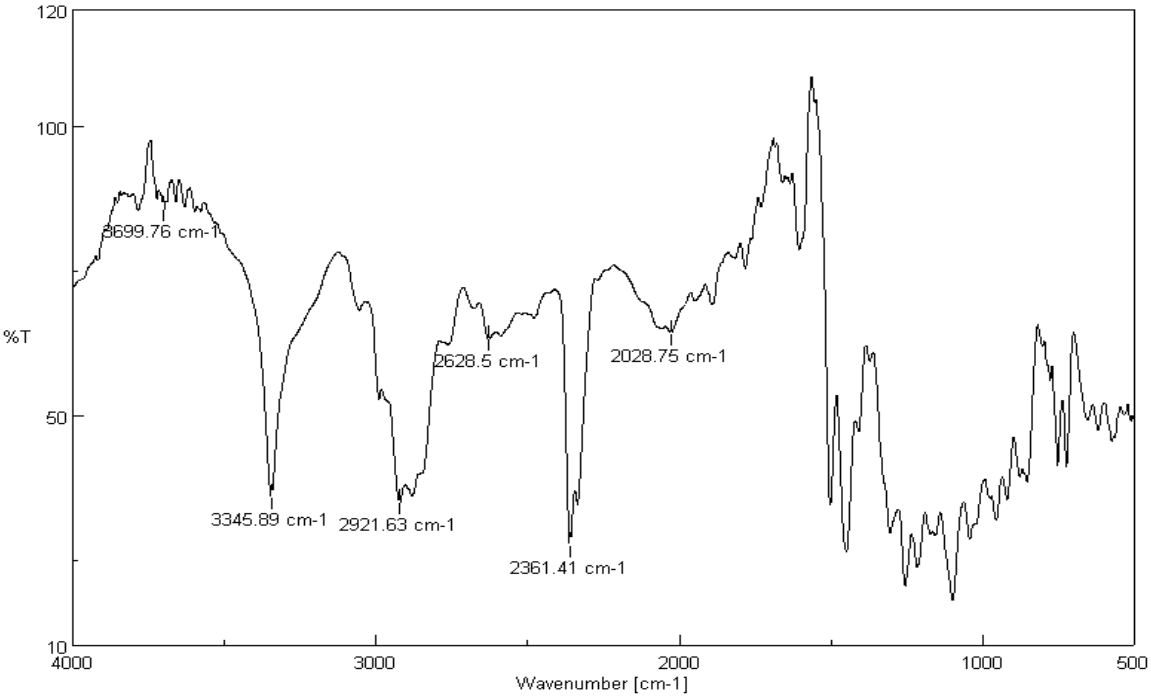


Figure15: IR spectrum of repaglinide-HPMC mixture

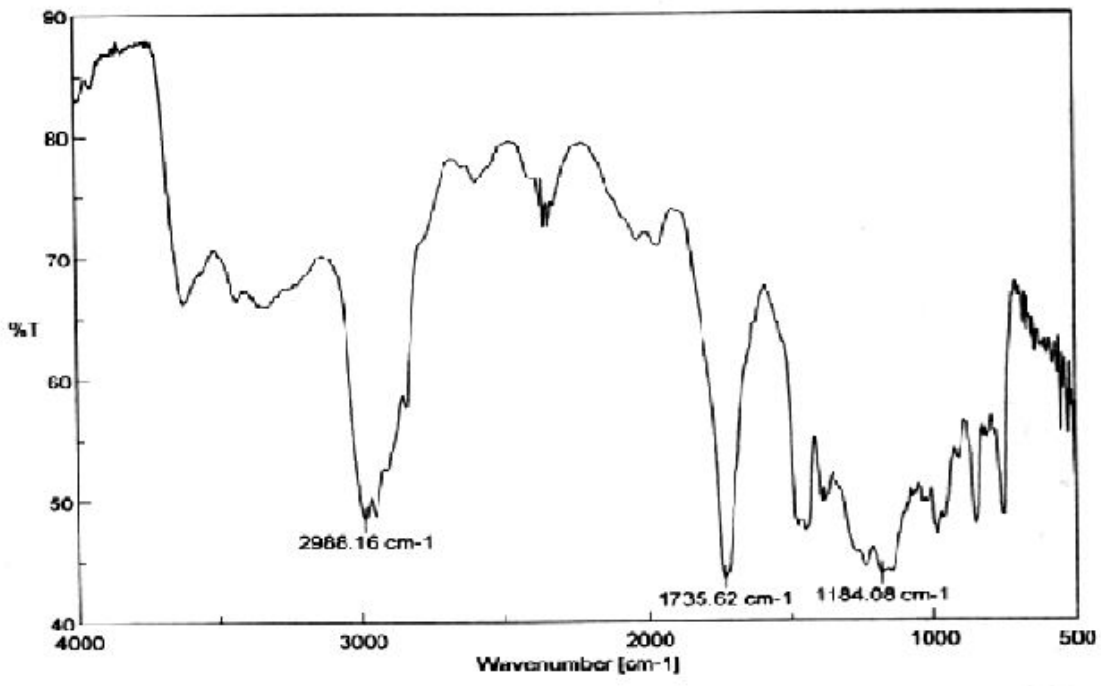


Figure 16: IR spectrum of ethyl cellulose pure

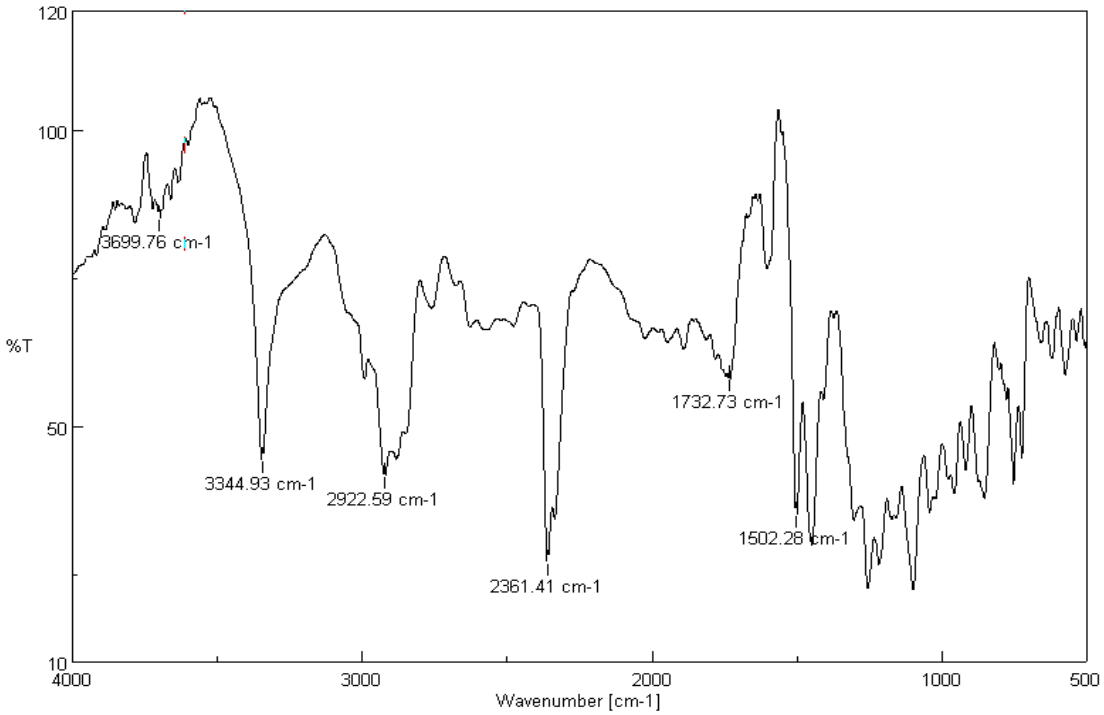


Figure 17: IR spectrum of repaglinide – ethyl cellulose mixture

EVALUATION OF FILMS

Thickness

With the help of screw gauze, the thickness of films was measured and the average thickness was noted. The thickness results are given in Table 9. The result indicates that there was no much difference in the thickness within the formulations. The order of the thickness of films is $F_3 < F_2 < F_1 < F_5 < F_4$

Table 9: Thickness uniformity of F_1 to F_5 film formulations

Sl. no.	Formulation code	Average Thickness (mm)			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	F_1	0.222	0.227	0.224	0.224 ± 0.002
2	F_2	0.219	0.221	0.223	0.221 ± 0.002
3	F_3	0.218	0.215	0.217	0.217 ± 0.002
4	F_4	0.296	0.291	0.297	0.295 ±0.003
5	F_5	0.284	0.281	0.285	0.283 ± 0.002

*Standard deviation, *n = 3*

Weight Uniformity

Drug loaded patches (1 x 1 cm^2) were tested for uniformity of weight and the results of weight uniformity are given in Table 11. Lesser S.D. values indicate that the patches were uniform. The order of the weight of films is $F_3 < F_2 < F_1 < F_5 < F_4$. This is in agreement with the uniformity of the thickness. Perusal to Table 11 indicates that patch F_4 exhibited highest weight. It could be because of the reason explained in the thickness uniformity.

Table 10: Weight uniformity data of F_1 to F_5 patch formulations

Sl. no.	Formulation code	Average weight (mg) Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	F_1	20.73	20.26	19.48	20.15 ± 0.396
2	F_2	18.29	18.30	19.18	18.59 ± 0.243
3	F_3	17.32	17.89	18.12	17.77 ± 0.264
4	F_4	23.41	22.39	22.63	22.81 ± 0.160
5	F_5	21.78	22.56	21.42	21.92 ± 0.416

*Standard deviation, *n = 3*

Swelling studies of the patches: The swelling of the drug loaded patches of size 1 x 1 cm^2 was studied up to 30 min in case of change in weight and 60 min in case of change in area. The swelling of the patches were observed in phosphate buffer solution (pH 7.4). The data for increase in weight due to swelling are given in Tables 11 to 15 for patches F_1 to F_5, respectively. The entire data are shown in Figure 18. Swelling was more pronounced in patch F_4 and F_5 which contain more of HPMC (hydrophilic polymer). Patches F_1 and F_2 showed lesser swelling (weight basis), may be due to the presence of higher concentrations of Ethyl cellulose hydrophobic polymer. The order of patches for their increase in weight due to swelling is $F_1 < F_2 < F_3 < F_4 < F_5$. Further, it should be verified with increase in area due to swelling.

Table11: Swelling studies Repaglinide of patch F_1 -Change in weight

Sl. no.	Time (min)	Weight of patch (mg)	Increase in weight (mg)	Percent increase
1	0	20.151 ± 0.394	0	0
2	5	27.856 ± 1.512	7.707 ± 1.889	38.231 ± 3.325
3	10	38.728 ± 2.168	18.577 ± 2.663	92.178 ± 2.040
4	15	43.748 ± 2.378	23.597 ± 1.927	117.108 ± 3.065(
5	20	50.174 ± 2.219	30.023 ± 1.786	148.990 ± 3.043(
6	25	59.262 ± 4.705	39.111 ± 4.257	194.089 ± 13.05(
7	30	64.393 ± 2.252	44.242 ± 1.821	219.552 ± 2.478(

* Each reading was an average of three determinations

Table12: Swelling studies of repaglinide patch F_2 -Change in weight

Sl. no.	Time (min)	Weight of patch (mg) AM ± S.D.	Increase in weight (mg) AM ± S.D.	Percent increase in weight* AM ± S.D.
1	0	18.597 ± 0.243	0.000	0.000
2	5	28.148 ± 3.757	9.551 ± 1.613	51.357 ± 15.403
3	10	35.623 ± 2.240	17.026 ± 2.191	91.552 ± 9.608
4	15	44.948 ± 2.755	26.343 ± 2.605	141.857 ± 9.894
5	20	51.622 ± 2.325	33.025 ± 2.370	177.582 ± 11.747
6	25	60.962 ± 2.404	42.365 ± 2.306	227.805 ± 9.076
7	30	62.034 ± 3.112	43.437 ± 3.262	233.569 ± 17.673

* Each reading was an average of three determinations

Table 13: Swelling studies of repaglinide patch F_3 - Change in weight

Sl. no.	Time (min)	Weight of patch (mg) AM ± S.D.	Increase in weight (mg) AM ± S.D.	Percent increase in weight* AM ± S.D.
1	0	17.771 ± 0.797	0.000	0.000
2	5	22.505 ± 2.685	4.734 ± 2.317	26.630 ± 12.295
3	10	30.845 ± 1.733	13.074 ± 1.601	73.567 ± 11.246
4	15	39.543 ± 3.504	21.772 ± 3.053	122.518 ± 15.048
5	20	45.198 ± 4.402	27.427 ± 4.156	154.338 ± 23.294
6	25	58.207 ± 3.263	40.436± 2.748	227.533 ± 13.295
7	30	69.474 ± 2.979	51.703 ± 2.506	290.945 ± 13.882

* Each reading was an average of three determinations.

Table 14: Swelling studies of repaglinide patch F_4 - Change in weight

Sl. no.	Time (min)	Weight of patch (mg) AM ± S.D.	Increase in weight (mg) AM ± S.D.	Percent increase in weight* AM ± S.D.
1	0	22.813 ± 0.359	0.000	0.000
2	5	49.214 ± 0.509	26.401 ± 0.444	115.728 ± 4.136
3	10	64.570 ± 1.534	41.757 ± 1.955	183.040 ± 5.955
4	15	86.870 ± 1.850	64.057 ± 1.447	280.791 ± 4.220
5	20	99.026 ± 1.904	76.213 ± 1.503	334.077 ± 4.469
6	25	110.227 ± 1.192	87.414 ± 1.050	383.176 ± 9.435
7	30	128.673 ± 2.385	105.86 ± 1.979	464.336 ± 5.425

* Each reading was an average of three determinations.

Table 15: Swelling studies of Repaglinide patch F_5 - Change in weight

Sl. no.	Time (min)	Weight of patch (mg) AM ± S.D.	Increase in weight (mg) AM ± S.D.	Percent increase in weight* AM ± S.D.
1	0	21.926 ± 0.426	0.000	0.000
2	5	45.238 ± 1.424	23.312 ± 1.848	106.321 ± 10.555
3	10	60.567 ± 2.127	38.641 ± 2.550	176.233 ± 14.635
4	15	74.282 ± 2.457	52.356 ± 2.853	238.785 ± 16.451
5	20	88.614 ± 0.747	66.688 ± 0.445	304.150 ± 4.202
6	25	110.160 ± 1.062	88.234 ± 0.776	402.417 ± 4.636
7	30	130.241 ± 1.337	108.31 ±1.456	493.992 ± 10.509

* Each reading was an average of three determinations.

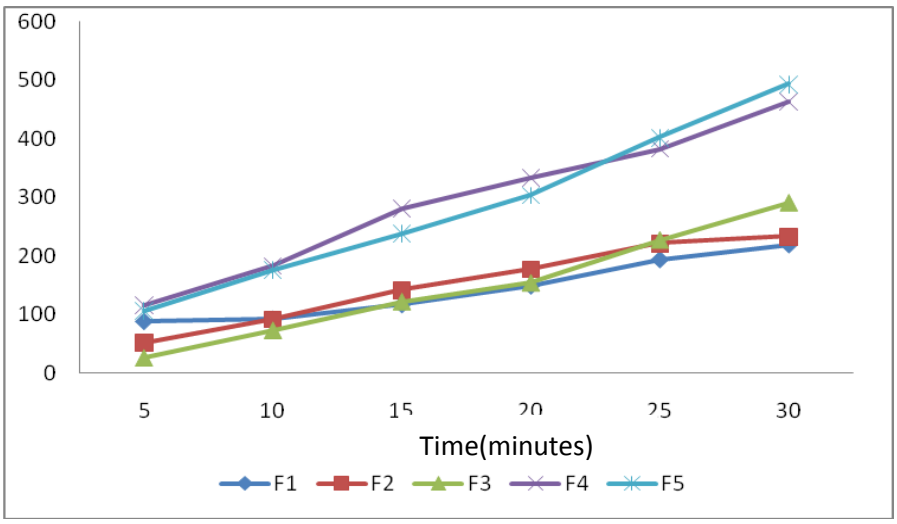


Figure 18: Swelling studies of repaglinide patches - Change in weight in phosphate buffer, pH 7.4.

The data for the increase in area due to swelling are given in Tables 11 to 15, respectively for patches F_1 to F_5. The data are shown

in Figure 18. Swelling was more pronounced in patches F_5 and F_4 which contain more of HPMC, which is hydrophilic polymer. Patches F_1 and F_2 showed lesser increase in area due to swelling. This must be due to the presence of higher concentrations of Ethyl cellulose. The order of patches for their increase in area due to swelling is $F_1 < F_3 < F_2 < F_4 < F_5$. This was in agreement with the increase in weight due to swelling.

Table 16: Swelling studies of repaglinide patch F_1 - Changes in area

Sl. no.	Time (min)	Area of patch cm^2 AM ± S.D.	Increase in area, cm^2 AM ± S.D.	Percent increase in area* AM ± S.D.
1	0	1	0	0
2	5	1.0231 ± 0.015	0.0231 ± 0.015	2.3100 ± 1.472
3	10	1.0435 ± 0.034	0.0435 ± 0.034	4.3533 ± 3.438
4	15	1.0501 ± 0.015	0.0501 ± 0.015	5.0100 ± 1.524
5	20	1.0679 ± 0.031	0.0679 ± 0.031	6.7900 ± 3.066
6	25	1.0717 ± 0.016	0.0717 ± 0.016	7.1733 ± 1.582
7	30	1.0871 ± 0.027	0.0871 ± 0.027	8.7167 ± 2.680
8	35	1.0923 ± 0.066	0.0923 ± 0.066	9.2367 ± 6.640
9	40	1.1111 ± 0.089	0.1111 ± 0.089	11.1167 ± 8.905
10	45	1.1210 ± 0.093	0.1210 ± 0.093	12.1033 ± 9.276
11	50	1.1321 ± 0.061	0.1321 ± 0.061	13.2167 ± 6.130
12	55	1.1428 ± 0.054	0.1428 ± 0.054	14.2800 ± 5.387
13	60	1.1550 ± 0.018	0.1550 ± 0.018	15.5033 ± 1.830

* Each reading was an average of three determinations.

Table 17: Swelling studies of repaglinide patch F_2-Changes in area

Sl. no.	Time (min)	Area of patch cm^2 AM ± S.D.	Increase in area, cm^2 AM ± S.D.	Percent increase in area* AM ± S.D.
1	0	1	0	0
2	5	1.0207 ± 0.026	0.0207 ± 0.026	2.0700 ± 2.565
3	10	1.0596 ± 0.047	0.0596 ± 0.047	5.9600 ± 4.704
4	15	1.0801 ± 0.015	0.0801 ± 0.015	8.0100 ± 1.524
5	20	1.1067 ± 0.015	0.1067 ± 0.015	10.6700 ± 1.542
6	25	1.1236 ± 0.016	0.1236 ± 0.016	12.3600 ± 1.559
7	30	1.1384 ± 0.016	0.1384 ± 0.016	13.8400 ± 1.593
8	35	1.1464 ± 0.043	0.1464 ± 0.043	14.6400 ± 1.350
9	40	1.1558 ± 0.050	0.1558 ± 0.050	15.5800 ± 2.006
10	45	1.1613 ± 0.045	0.1613 ± 0.045	16.1300 ± 1.464
11	50	1.1705 ± 0.051	0.1705 ± 0.051	17.0500 ± 1.115
12	55	1.1854 ± 0.062	0.1854 ± 0.062	18.5400 ± 1.237
13	60	1.1915 ± 0.078	0.1915 ± 0.078	19.1500 ± 2.753

* Each reading was an average of three determinations.

Table 18: Swelling studies of Repaglinide patch F_3 - Changes in area

Sl. no.	Time (min)	Area of patch cm^2 AM ± S.D.	Increase in area, cm^2 AM ± S.D.	Percent increase in area* AM ± S.D.
1	0	1	0	0
2	5	1.0336 ± 0.015	0.0336 ± 0.015	3.3600 ± 1.472
3	10	1.0852 ± 0.030	0.0852 ± 0.030	8.5200 ± 2.996
4	15	1.1379 ± 0.031	0.1379 ± 0.031	13.7900 ± 3.066
5	20	1.2103 ± 0.047	0.2103 ± 0.047	21.0367 ± 4.734
6	25	1.2563 ± 0.016	0.2563 ± 0.016	25.6367 ± 1.617
7	30	1.2830 ± 0.017	0.2830 ± 0.017	28.3067 ± 1.651
8	35	1.3112 ± 0.034	0.3112 ± 0.034	31.1233 ± 3.354
9	40	1.3547 ± 0.007	0.3547 ± 0.007	35.4700 ± 0.710
10	45	1.3844 ± 0.010	0.3844 ± 0.010	38.4467 ± 0.993
11	50	1.4300 ± 0.017	0.4300 ± 0.017	43.0033 ± 1.726
12	55	1.4601 ± 0.017	0.4601 ± 0.017	46.0167 ± 1.738
13	60	1.4710 ± 0.035	0.4710 ± 0.035	47.1067 ± 3.539

* Each reading was an average of three determinations.

Table 19: Swelling studies of repaglinide patch F_4 - Changes in area

Sl. no.	Time (min)	Area of patch cm^2 AM ± S.D.	Increase in area, cm^2 AM ± S.D.	Percent increase in area* AM ± S.D.
1	0	1	0	0
2	5	1.0336 ± 0.015	0.0336 ± 0.015	3.3600 ± 1.472
3	10	1.0592 ± 0.015	0.0592 ± 0.015	5.9200 ± 1.490
4	15	1.1115 ± 0.040	0.1115 ± 0.040	11.1500 ± 1.036
5	20	1.1379 ± 0.031	0.1379 ± 0.031	13.7900 ± 2.066
6	25	1.1756 ± 0.018	0.1756 ± 0.018	17.5600 ± 1.758
7	30	1.1856 ± 0.018	0.1856 ± 0.018	18.5600 ± 1.758
8	35	1.2103 ± 0.047	0.2103 ± 0.047	21.0267 ± 1.734
9	40	1.2693 ± 0.043	0.2693 ± 0.043	26.9367 ± 2.311
10	45	1.3065 ± 0.043	0.3065 ± 0.043	30.6500 ± 1.268
11	50	1.3847 ± 0.043	0.3847 ± 0.043	38.4667 ± 2.320
12	55	1.4929 ± 0.058	0.4929 ± 0.058	49.2900 ± 1.750
13	60	1.6223 ± 0.077	0.6223 ± 0.077	62.2300 ± 1.658

* Each reading was an average of three determinations.

Table 20: Swelling studies of repaglinide patch F_5 -Changes in area

Sl. no.	Time (min)	Area of patch cm^2 AM ± S.D.	Increase in area, cm^2 AM ± S.D.	Percent increase in area* AM ± S.D.
1	0	1	0	0
2	5	1.0421 ± 0.015	0.0421 ± 0.015	4.2100 ± 1.472
3	10	1.1113 ± 0.015	0.1113 ± 0.015	11.1300 ± 1.524
4	15	1.1726 ± 0.017	0.1726 ± 0.017	17.2567 ± 1.738
5	20	1.2476 ± 0.017	0.2476 ± 0.017	24.7600 ± 1.732
6	25	1.2851 ± 0.015	0.2851 ± 0.015	28.5133 ± 1.518
7	30	1.3419 ± 0.034	0.3419 ± 0.034	34.1867 ± 3.354
8	35	1.3752 ± 0.046	0.3752 ± 0.046	37.5167 ± 4.641
9	40	1.4003 ± 0.017	0.4003 ± 0.017	40.0267 ± 1.703
10	45	1.4600 ± 0.017	0.4600 ± 0.017	46.0033 ± 1.726
11	50	1.5901 ± 0.017	0.5901 ± 0.017	59.0167 ± 1.738
12	55	1.6707 ± 0.031	0.6707 ± 0.031	67.0700 ± 3.065
13	60	1.7811 ± 0.018	0.7811 ± 0.018	78.1133 ± 1.778

* Each reading was an average of three determinations.

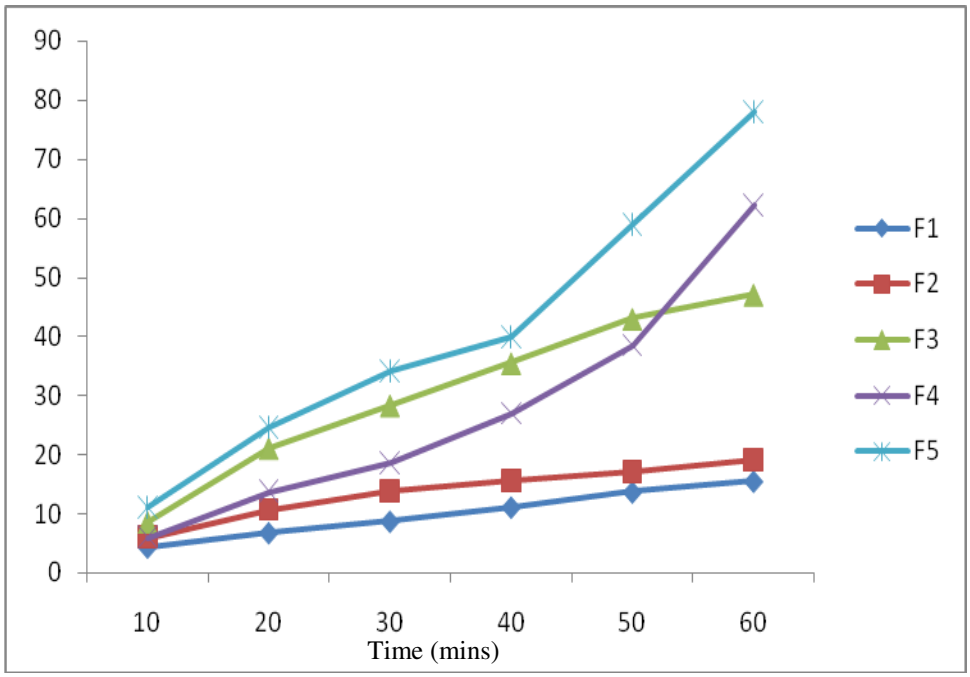


Figure 19:Swelling studies of repaglinide patches - Change in area in phosphate buffer, pH 7.4.

Tensile Strength of Films

Tensile strength was determined using Hounse Field universal testing machine for drug-loaded films. The results (average of 3 determinations) are given in the Table 26 and the graph is represented in the Figure 22.The order of tensile strength of the films is $F_1 < F_2 < F_3 < F_4 < F_5$. The tensile strengths of drug loaded films were higher than dummy films. This is justified because dissolved repaglinide strengthened the bonding of polymer chains. With increase in HPMC proportion the tensile strength of films was increased. It reflects that the soluble polymer develops cross linking better than insoluble polymer. This is in agreement with the viscosity determinations. More the solubility of the polymer higher will be the tensile strength.

Table 21: Data of tensile strength of F_1 to F_5 formulation

S.D. = Standard deviation, n = 3

S.No.	Film		Tensile strength (Kg ± S.D.)	Extension in length(mm)
1	F_1	Blank	0.5262 ± 0.0431	2.04±0.03
		Drug	0.6632 ± 0.0342	12.71±0.04
2	F_2	Blank	0.597 ± 0.0128	5.46±0.02
		Drug	0.717 ± 0.0170	7.14±0.03
3	F_3	Blank	1.233 ± 0.265	2.10±0.04
		Drug	1.473 ± 0.220	7.66±0.02
4	F_4	Blank	1.416 ±0.306	11.25±0.03
		Drug	1.749 ±0.216	15.79±0.02
5	F_5	Blank	1.660 ± 0.265	1.61±0.02
		Drug	1.956 ±0.147	2.10±0.03

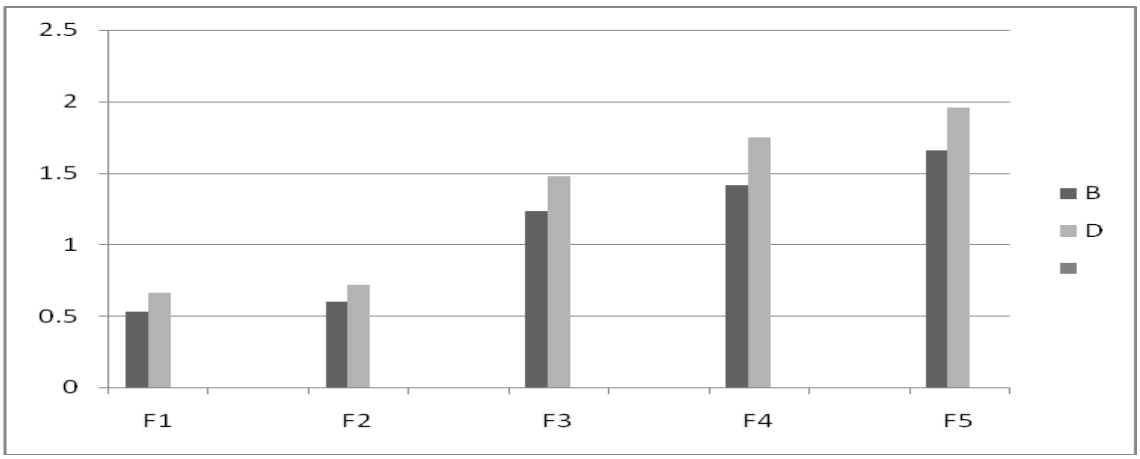


Figure 20: Tensile strength of films determined using Hounse Field universal testing machine for the blank and drug loaded films.

Folding Endurance

The recorded folding endurance of the films was > 300 times. It means all formulations had good film properties.

Water Vapour Transmission Rate (WVTR)

The water vapour transmission rates of different formulations were evaluated and the results are shown in Table 22.

Repaglinide patches containing HPMC alone showed higher WVTR as compared to the formulations containing Ethyl cellulose. This may be due to the HPMC, which is more hydrophilic in nature than Ethyl cellulose, which is less permeable to water vapour. Formulation F_5 showed highest WVTR where as F_1 showed lowest WVTR.

Table 22: Water vapour transmission rate of F_1 to F_5 formulations

Sl. No.	Formulation Code	Water vapour transmission rate (WVTR) Mean ± S.D.*x 10^{-3}
1	F_1	0.142 ± 0.065
2	F_2	0.151 ± 0.039
3	F_3	0.207 ± 0.029
4	F_4	0.312 ± 0.018
5	F_5	0.378 ± 0.021

*Standard deviation, n = 3

Drug Content Uniformity

Drug content of the film was carried out to ascertain that the drug is uniformly distributed into the formulation. The film of size 2 ×

2 cm^2 was cut and placed in a volumetric flask. Ten ml of methanol was added and the contents were stirred in a shaker bath for 24 h to dissolve the film. Subsequent dilutions were made with phosphate buffer (pH 7.4). The absorbance of the solution was measured against the corresponding blank solution at 237 nm using UV-VIS spectrophotometer. The results obtained are represented in the Table 23.

From the results obtained (i.e., lowest S.D. values), it was clear that there was proper distribution of repaglinide in the film formulations. Hence it was concluded that drug was uniformly distributed in all the formulations.

Table 23: Drug content of F_1 to F_5 formulations

Sl. No.	Formulation code	Drug content (mg)			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	F_1	1.267	1.372	1.305	1.294 ± 0.0531
2	F_2	1.201	1.147	1.399	1.279 ± 0.0807
3	F_3	1.223	1.298	1.333	1.290 ± 0.081
4	F_4	1.278	1.302	1.291	1.310 ± 0.0451
5	F_5	1.334	1.258	1.226	1.391 ± 0.0887

***Standard deviation, *n* = 3**

Dose calculation

The total dose of repaglinide for one daily sustained release formulation was calculated by following equation using the available pharmacokinetic data

$D_T = D_{IR} (1+0.693 * t / t_{1/2})$

D_T = Total dose of drug

D_{IR} = Dose of immediate release part

t = Time during which (S R) is desired (24 hours)

$t_{1/2}$= Half life of drug (3hours)

$$\mathbf{C_{ss}} = \frac{\text{F * total dose}}{\mathbf{Cl_p} * \text{ j}} = \frac{0.7*16}{394 * 3} = 0.0094$$

$$\mathbf{D_{IR}} = \frac{\mathbf{C_{ss}} * Vd}{F} = \frac{0.0094 * 2 * 60}{0.7} = 1.16 \text{ mg}$$

C_{ss} = Steady state concentration of drug in plasma

Cl_p = Plasma clearance of drug

J = Frequency of dosing

Vd = Volume of dug distribution

F = Fraction of drug absorbed

(Cl_p, j, Vd and F are obtained from literature)

Thus D_T= 1.61(1+ 0.693 * 24/3)

D_T= 10.54 is equivalent to = 10 mg

According to the theoretical release pattern, once daily repaglinide sustained release formulation should release 1.61 mg in one hour, 0.365mg every hour and 100% in 24 hours.

Table 24: Dissolution profile of repaglinide according to theoretical calculation is as follows

Sampling time(h)	%Drug release
0	0
1	16.10
2	19.75
4	27.70
6	34.35
8	41.65
12	56.25
16	70.85
20	85.45
24	100.0

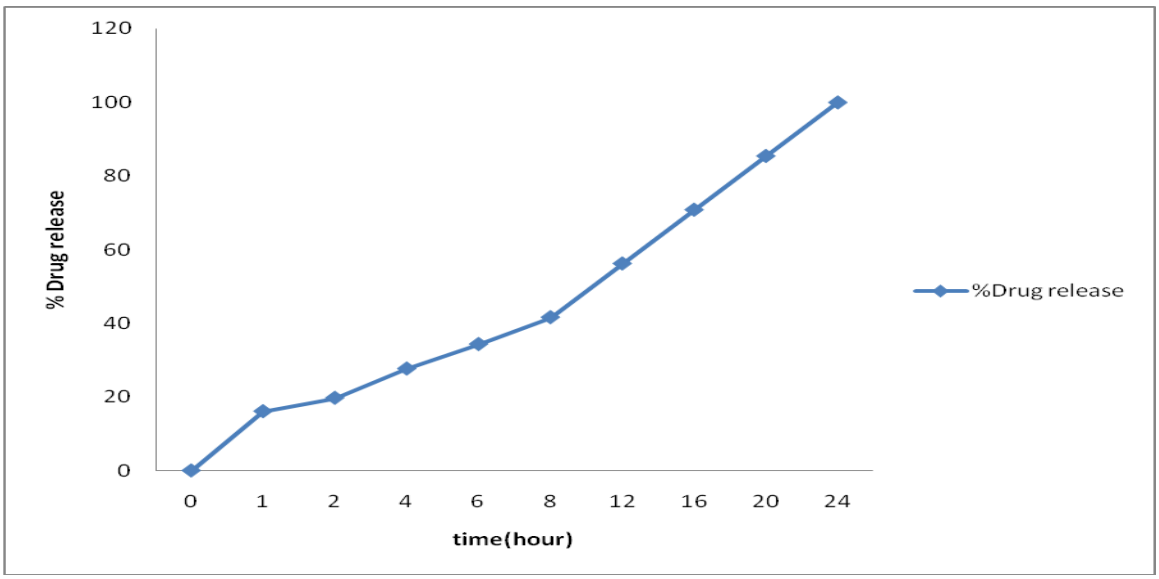


Figure 21 Dissolution profile of Repaglinide according to theoretical calculation

***In Vitro* Release Studies**

In vitro release studies of repaglinide films were carried out in phosphate buffer (pH 7.4). The release data of repaglinide are given in Tables25to 29, respectively for patches F_1 to F_5 repaglinide.

Table25: *In vitro* release of repaglinide from film F_1 in phosphate buffer (pH 7.4)

Time (hours)	Cumulative * drug released (mg) AM ± S.D	% Drug released	% Drug remain unreleased	log% drug remain unreleased
0	0	0	100	2
1	0.7550 ± 0.077	7.55	97.617	1.9895
2	0.9001 ±0.081	16.551	83.449	1.9214
4	1.0811 ±0.052	27.362	72.638	1.8611
6	1.1028 ±0.030	38.390	61.610	1.7895
8	1.3280 ± 0.042	42.476	57.524	1.7598
12	1.0166 ± 0.070	48.049	51.051	1.7080
16	1.0042 ± 0.040	55.495	44.505	1.6484
20	1.0094 ± 0.048	62.832	37.168	1.5701
24	1.0036 ± 0.110	69.684	30.316	1.4816

* Each reading is an average of three determinations.

Initial drug concentration = 0.7550mg

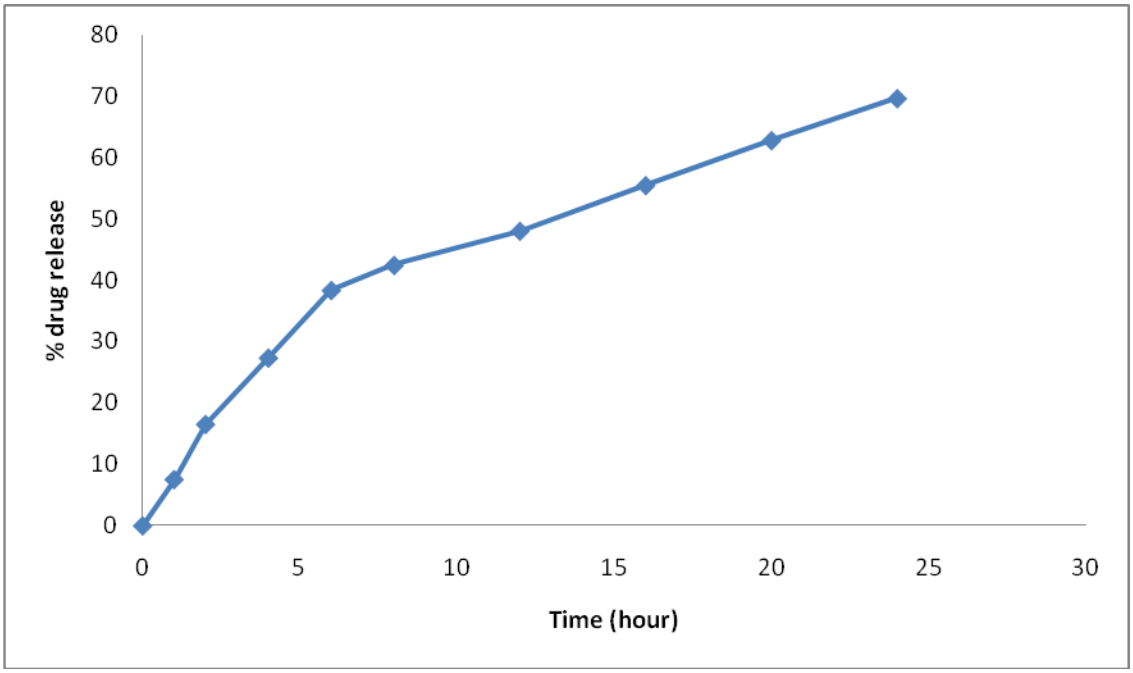


Figure 22: *In vitro* release of repaglinide from film F_1 in phosphate buffer (pH 7.4)

Table 26: *In vitro* release of Repaglinide from film F_2 in phosphate buffer (pH 7.4)

Time (min)	Cumulative * Drug released (mg) AM ± S.D.	% Drug Released	% Drug remain unreleased	Log % drug remain unreleased
0	0	0	100	2
1	0.2435 ± 0.030	2.435	45.6453	1.6593
2	0.5014 ± 0.113	5.014	43.9241	1.6427
4	0.7353 ± 0.071	7.353	39.8635	1.6005
6	1.0325 ± 0.069	18.325	35.9565	1.5557
8	1.1792 ± 0.083	24.948	33.0514	1.5191
12	1.3115 ±0.039	51.947	28.0521	1.4479
16	1.3629 ± 0.041	60.550	25.4498	1.4056
20	1.4020 ± 0.060	71.107	22.8920	1.3596
24	1.4992 ± 0.070	78.005	17.9947	1.2551

*Each reading was an average of three determinations

Initial drug concentration = 0.2435 mg.

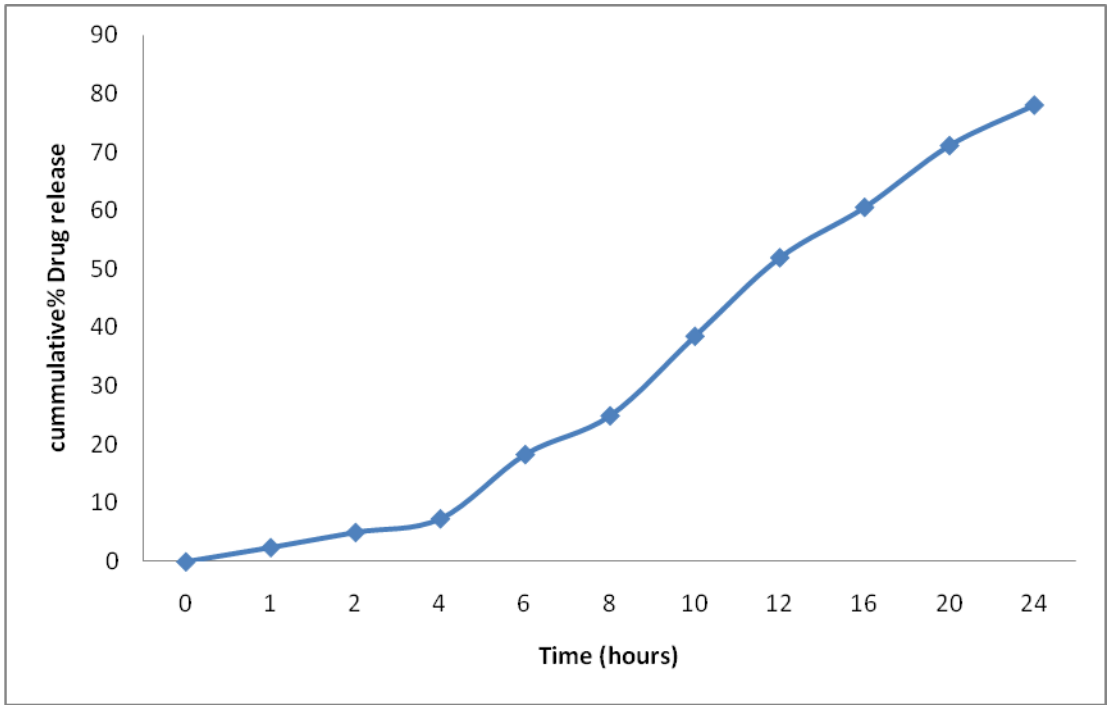


Figure 25:*In vitro* release of repaglinide from film F_2 in phosphate buffer (pH 7.4)

Table 27:*In vitro* release of repaglinide from film F_3 in phosphate buffer (pH 7.4)

Time (hours)	Cumulative * drug released (mg) AM ± S.D.	% Drug released	% Drug remain unreleased	log% drug remain unreleased
0	0	0	100	2
1	1.6014 ± 0.021	16.014	83.986	1.9242
2	0.3657± 0.017	20.564	78.436	1.8975
4	0.6921 ± 0.021	28.715	71.285	1.8529
6	0.7137 ± 0.017	34.361	65.639	1.8171
8	0.7443 ± 0.082	42.024	57.976	1.7632
12	0.7906 ± 0.035	56.544	43.456	1.6377
16	1.4261 ± 0.026	70.318	29.682	1.4724
20	1.4672 ± 0.035	85.244	14.756	1.1689
24	1.3224 ± 0.025	98.249	1.751	0.2432

*Each reading was an average of three determinations

Initial drug concentration= 1.6014 mg.

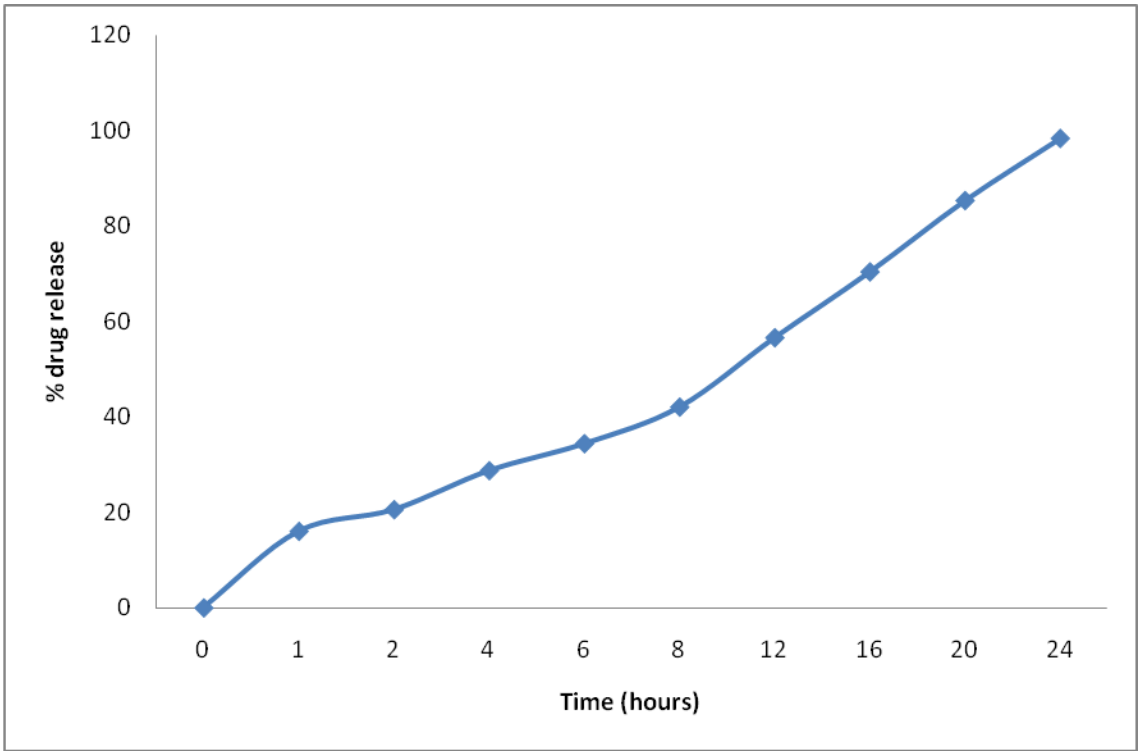


Figure 24:*In vitro* release of repaglinide from film F_3 in phosphate buffer (pH 7.4)

Table 28: *In vitro* release of repaglinide from film F_4 in phosphate buffer (pH 7.4)

Time (hours)	Cumulative * drug released (mg) AM ± S.D	% Drug released	% Drug remain unreleased	log% drug remain unreleased
0	0	0	100	2
1	0.7550 ± 0.077	7.550	92.450	1.9659
2	1.2001 ±0.081	12.870	87.130	1.9401
4	1.3811 ±0.052	24.087	75.913	1.8803
6	1.5028 ±0.030	45.251	54.785	1.7386
8	1.6280 ± 0.042	64.476	35.524	1.5504
12	1.7166 ± 0.070	77.049	22.951	1.3608
16	1.8542 ± 0.040	89.495	10.505	1.0213
20	1.9294 ± 0.048	99.000	1.000	0

*Each reading was an average of three determinations

Initial drug concentration = 0.7550mg

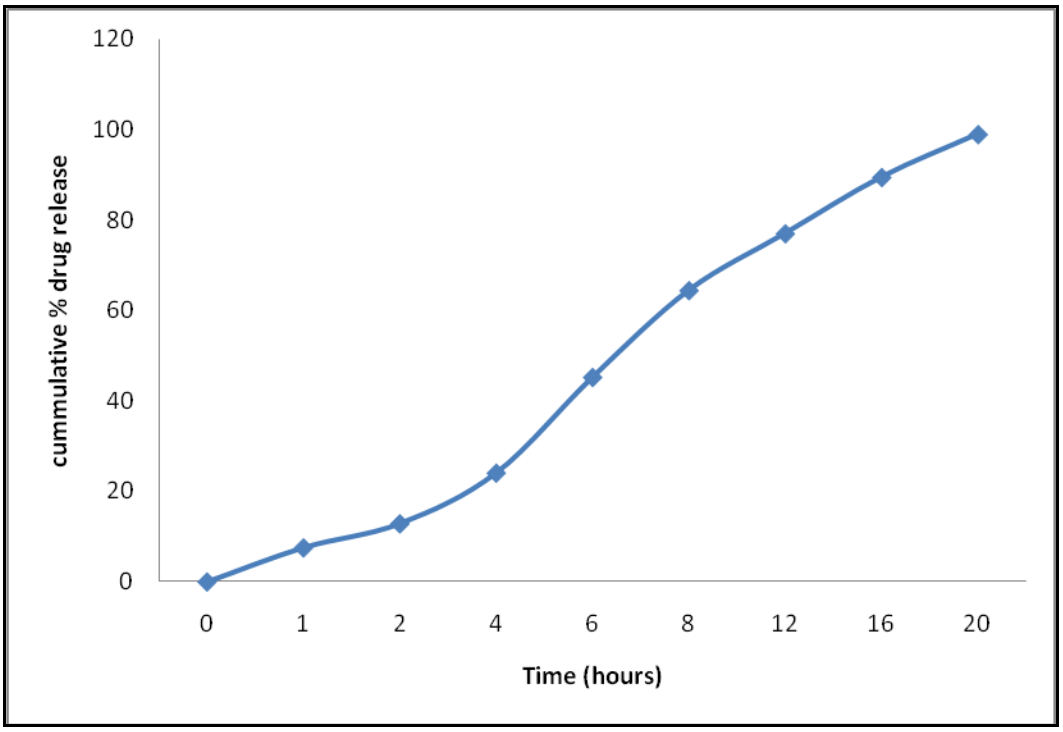


Figure 27:*In vitro* release of repaglinide from film F_4 in phosphate buffer (pH 7.4)

Table 29: *In vitro* release of repaglinide from film F_5 in phosphate buffer (pH 7.4)

Time (hours)	Cumulative drug* released (mg) AM ± S.D.	% Drug released	% Drug remain unreleased	log% drug remain unreleased
0	0.000 ±0.000	0	100	2.000
1	1.8209 ± 0.381	35.0982	64.9017	1.8122
2	2.5849 ± 0.417	48.2311	51.7689	1.7140
4	2.9029 ± 0.063	75.1650	24.8349	1.3950
6	3.1911 ± 0.042	89.5429	10.4571	1.0194
8	3.2573 ± 0.042	100	0	0

*Each reading was an average of three determinations

Initial drug concentration = 1.8209 mg.

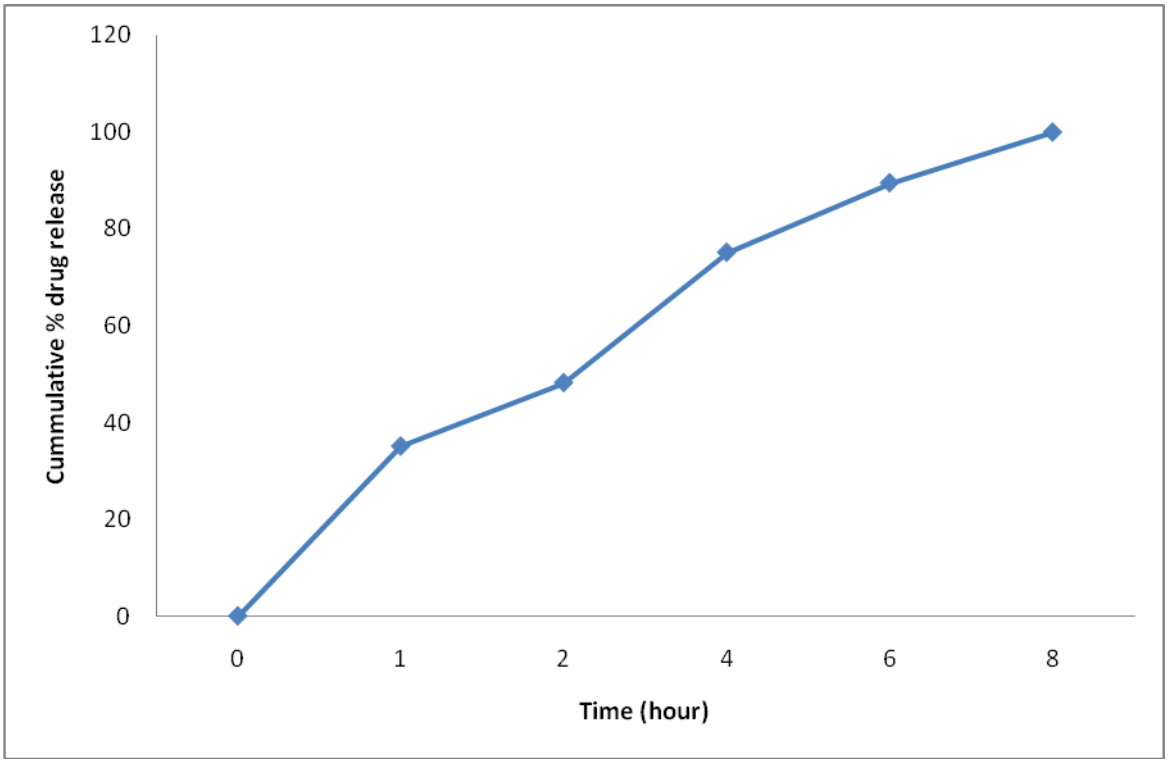


Figure 28:*In vitro* release of repaglinide from film F_5 in phosphate buffer (pH 7.4)

The *in vitro* release data for repaglinide from films are compiled in Table 30.

Table 30: **Compilation of *in vitro* release of repaglinide at 24 h**

Sl. No.	Film code	% Drug released
1	**F_1**	**69.68**
2	**F_2**	**78.00**
3	**F_3**	**98.24**
4	**F_4**	**99.00***
5	**F_5**	**100****

***20 h and ** 8 h**

A perusal to Figures 22 to 26, and Table 26 and 25 indicated that the release of Repaglinide was faster in films containing more concentration of HPMC.

Drug permeation profiles from formulations F_1 to F_5 are shown in Figure 30. Perusal to Figure 26 indicates that 100 % of drug was released within 8 h from F_5 and followed zero-order kinetics. This means the film has to be applied several times a day to maintain therapeutic levels constant. The faster drug release rate is due to the use of hydrophilic polymer (HPMC) alone. To get sustained release, copolymer that decreases the drug release rate is needed to be added. Therefore, rate-controlling membranes of Ethyl cellulose were cast with the aim to achieve controlled release of repaglinide from drug reservoirs of HPMC.

Perusal to Figure 23 indicates that, the cumulative amount of drug released after 24 h from F_2 was found to be 78.00 % when compared to F_1 the drug release from F_2 was increased from 20% . This effect was because of the copolymer, HPMC, which acted as the rate-controlling polymer. However, the target is to get drug release up to 24 h. Hence there is a need to delay the drug release further.

The sustained drug release could be achieved by increasing the copolymer concentration in the formulation by maintaining the total polymer concentration same i.e., 400 mg. In the formulations F_2 to F_5, HPMC was increased in order. From Figure 24, it was found that only 98.249% of repaglinide was released from F_3 at the end of 24 h.

In the formulations F_4 and F_5, an attempt was made by adding more concentration of HPMC to get the required release rate. In the formulation F_4 and F_5 the drug release was found to faster because of further increase in concentration of HPMC which is highly hydrophobic in nature.

In the formulation F_3, both HPMC and Ethyl cellulose was equally added. The drug release from this film was found to be 98.02%. When compared to F_1 and F_2, 20% drug release was improved. Hence, the use of HPMC was a successful attempt. But the thirst for improving the formulation continued to develop further.

All the formulations (F_1 to F_5) released drug in a biphasic pattern, i.e. a faster release that occurred for first hour, after which the release rate slowed down. This result can be explained from the theoretical point of view. Ethyl cellulose being an inert polymer,

solvent penetration into the film was rate–limiting factor for the release of the active principle. At the beginning of the process, the active substance at and near the surface of the film dissolves quickly. When the dissolution process advances, there is a greater resistance to the penetration of the solvent in the inside of the matrix film, due to the non-hydrophilicity of the polymer and the decreasing length of the solvent front. The drug, easily accessible by water immediately dissolves and diffuses from the interface between the film surface and surrounding media after which diffusion process slows down. The formulations can be arranged in order of release rate as: $F_1 < F_2 < F_3 < F_4 < F_5$.

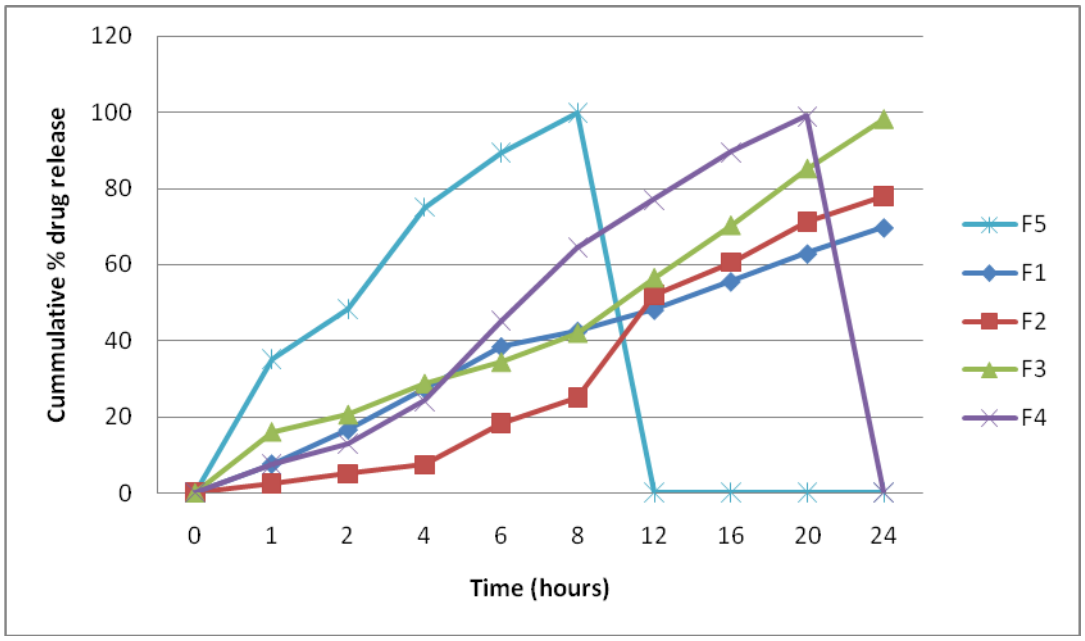


Figure 27:***In vitro*** **release of Repaglinide from films F_1 to F_5.**

Kinetics **of** drug release (Zero and First Order)

Zero order release

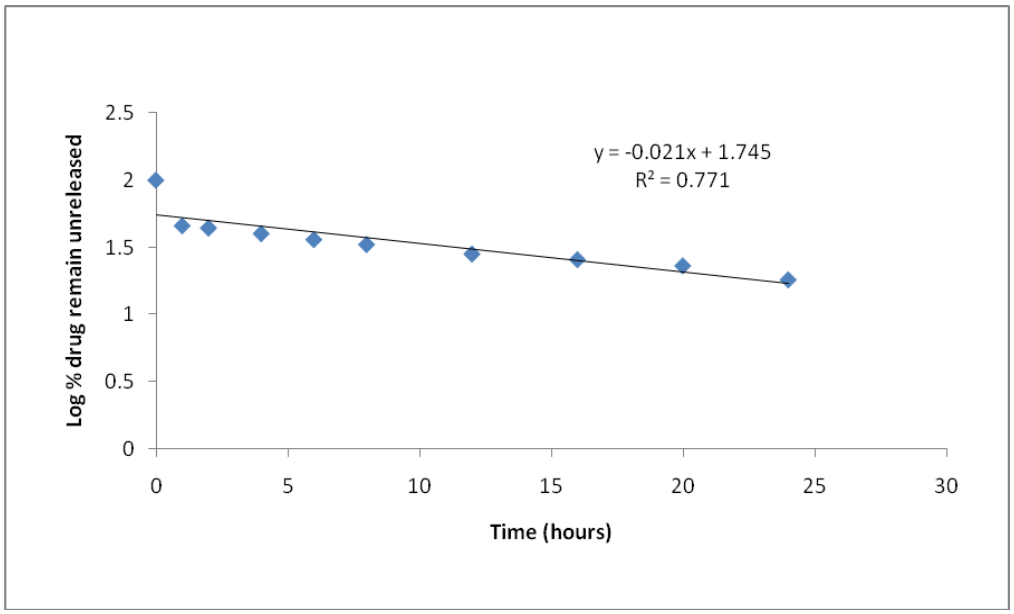


Figure 28:***In vitro*** **release of Repaglinide from film F_1 in phosphate buffer (pH 7.4).**

First order release

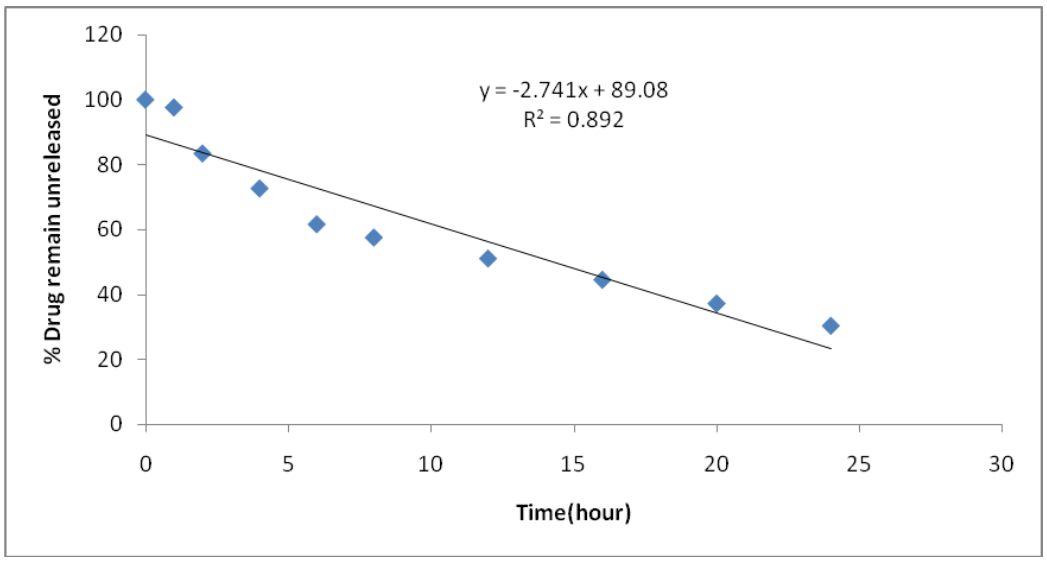


Figure 29:***In vitro*** release of Repaglinide from film F_1 in phosphate buffer (pH 7.4).

Zero order release

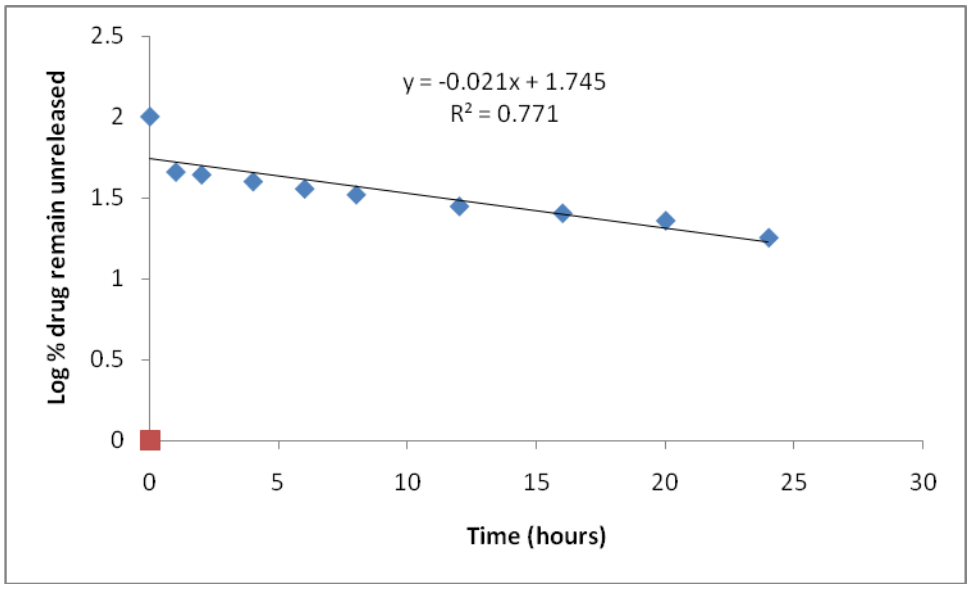


Figure 30:***In*** *vitro* release of Repaglinide from film F_2 in phosphate buffer (pH 7.4).

First order release

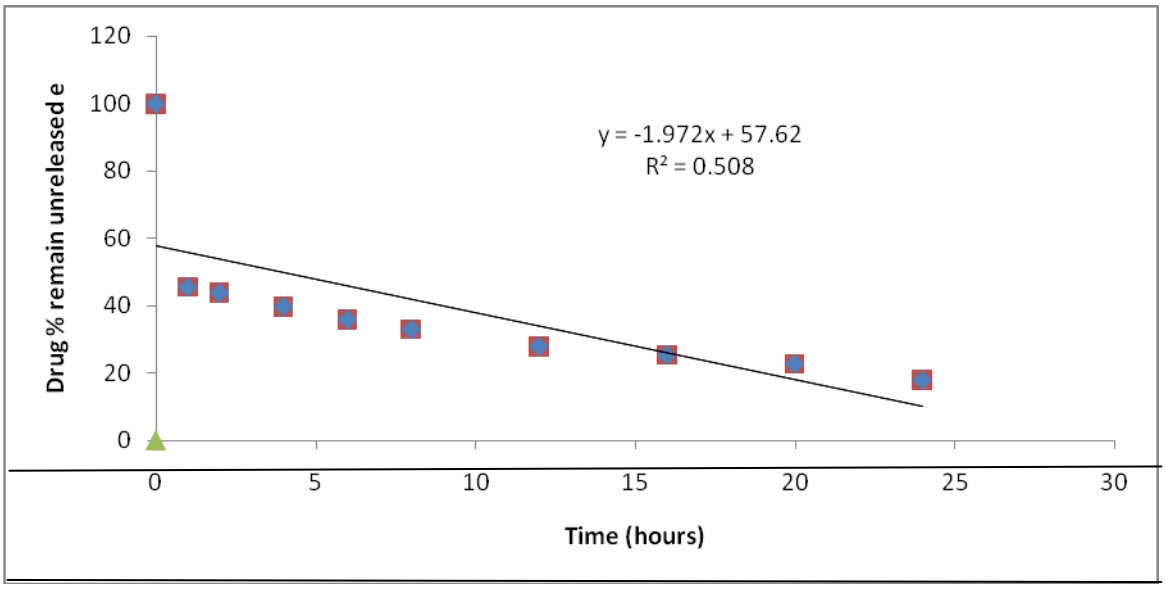


Figure 31:***In*** *vitro* release of Repaglinide from film F_2 in phosphate buffer (pH 7.4).

Zero order release

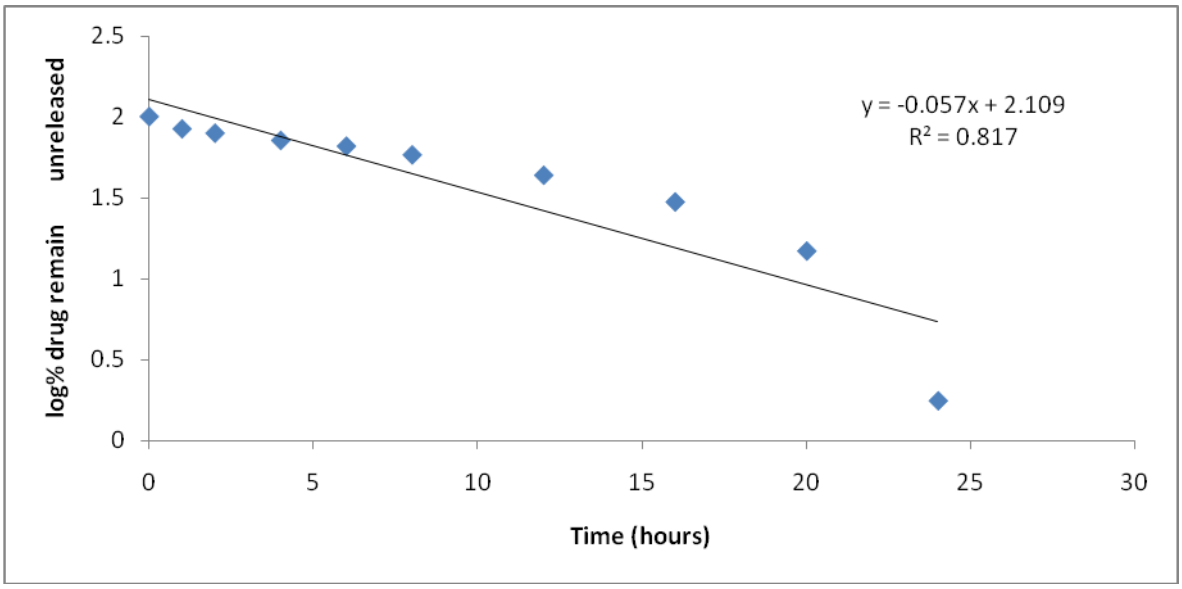


Figure 32:***In vitro*** release of Repaglinide from film F_3 in phosphate buffer (pH 7.4).

First order release

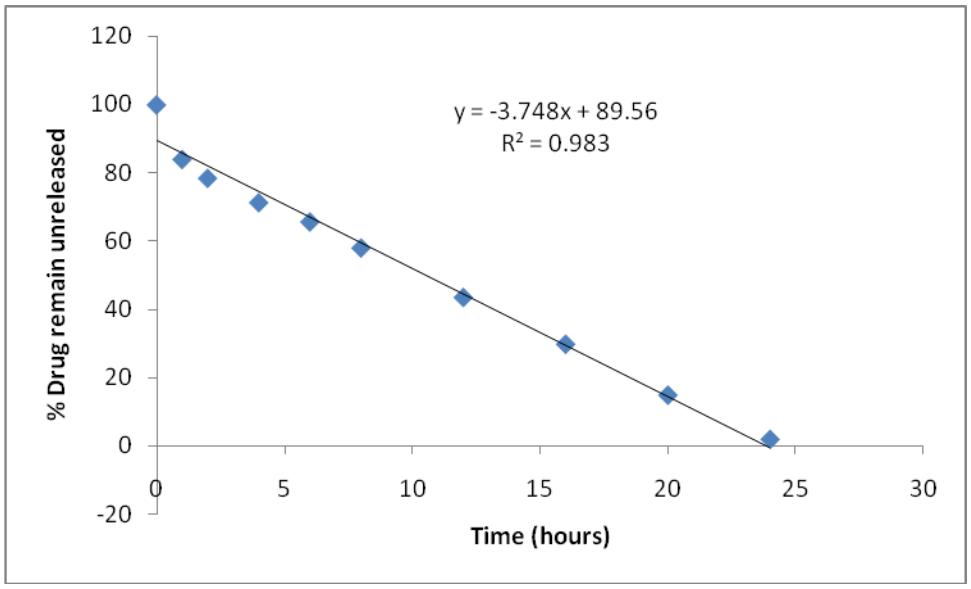


Figure 33:***In vitro*** release of Repaglinide from film F_3 in phosphate buffer (pH 7.4).

Zero order release

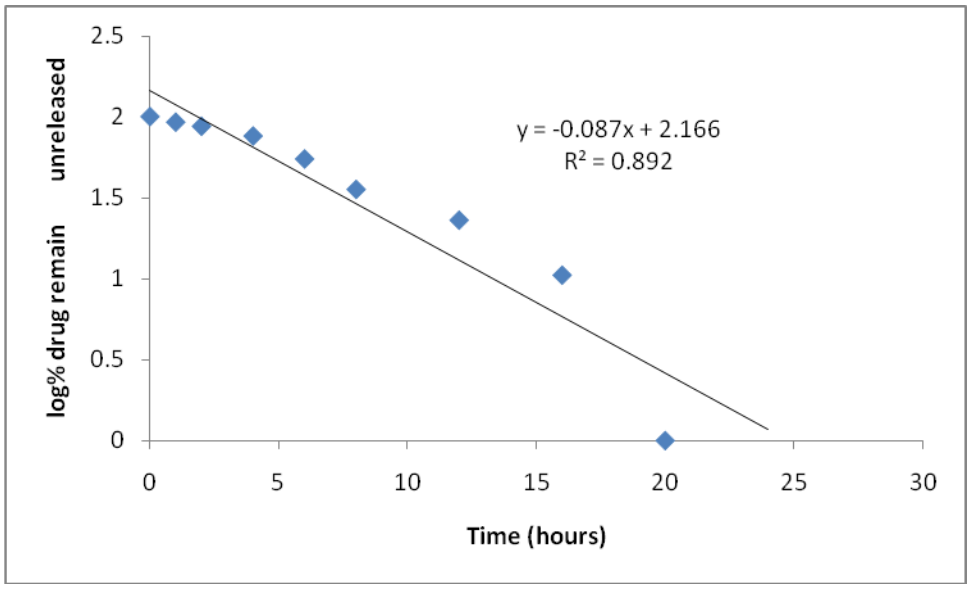


Figure 34:***In vitro*** release of Repaglinide from film F_4 in phosphate buffer (pH 7.4).

First order release

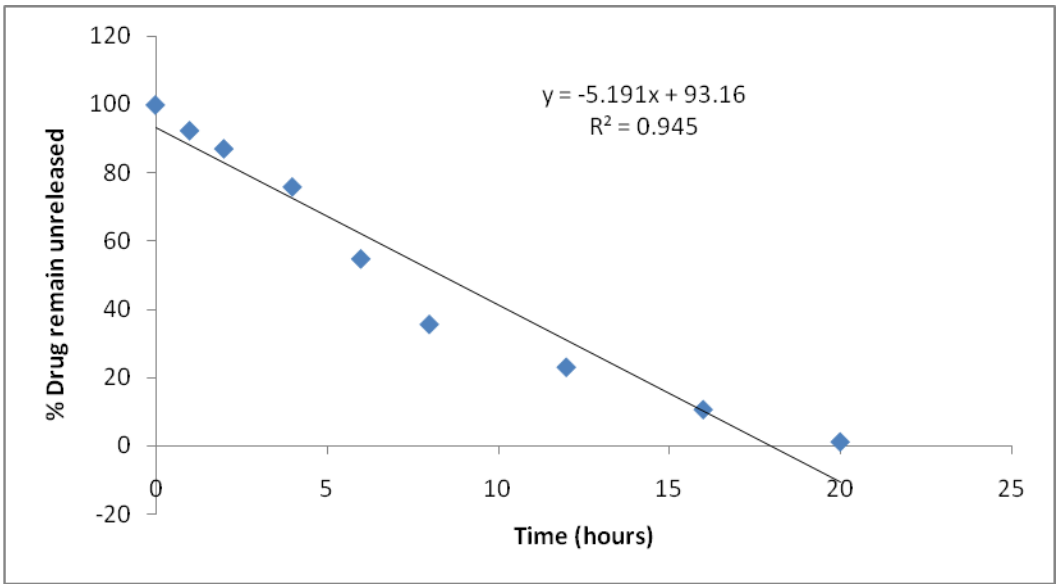


Figure 35:***In vitro*** release of Repaglinide from film F_4 in phosphate buffer (pH 7.4).

Zero order release

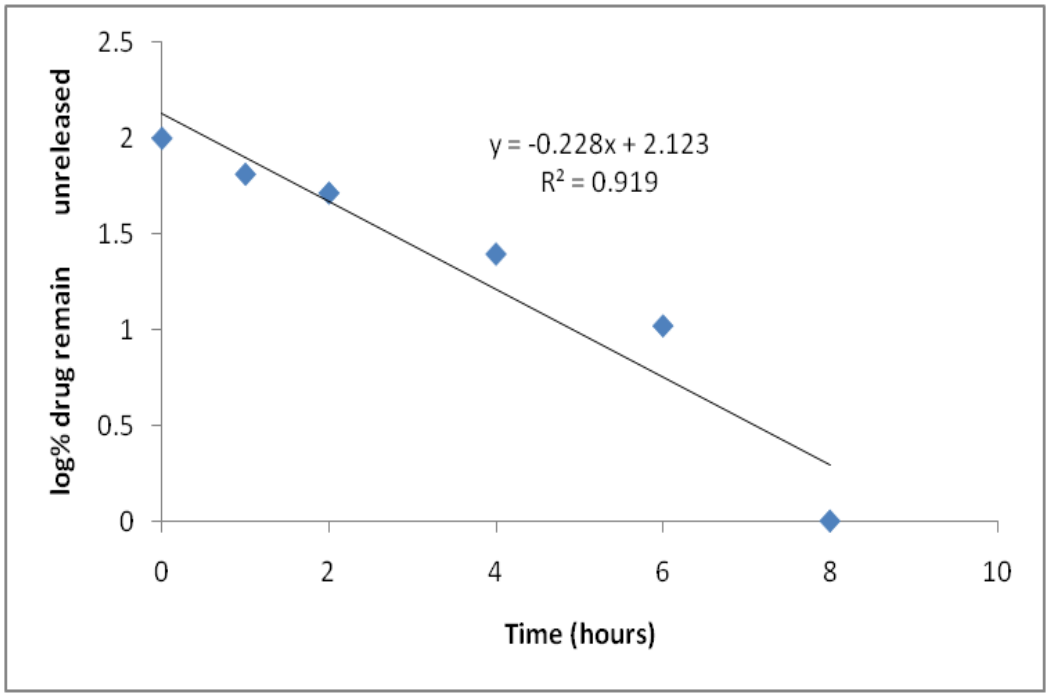


Figure 36:***In vitro*** release of Repaglinide from film F_5 in phosphate buffer (pH 7.4).

First order release

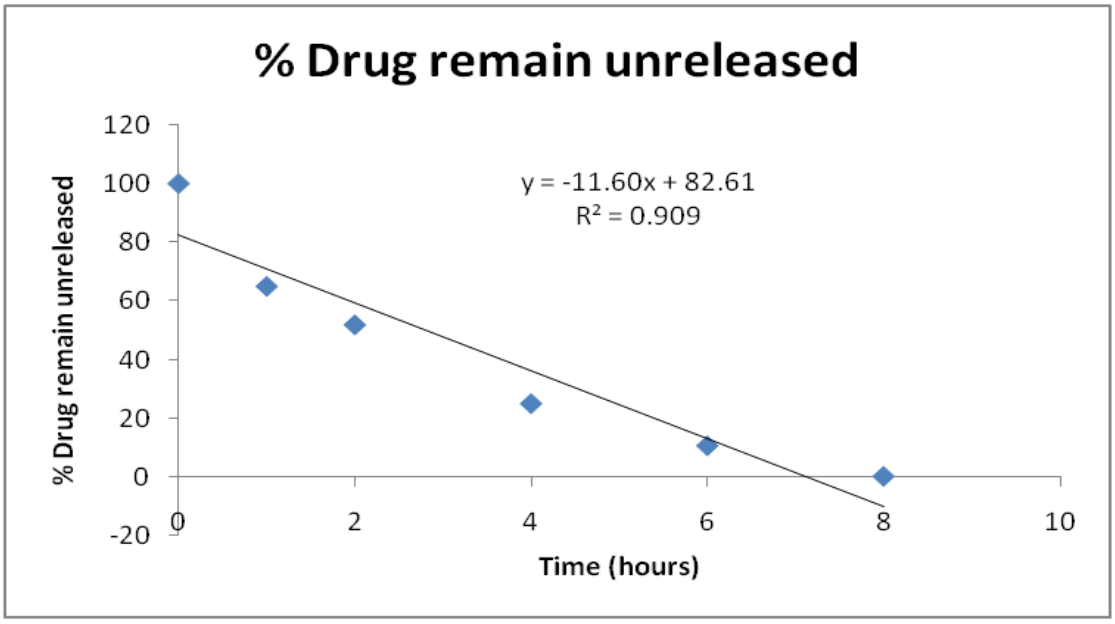


Figure 37:***In vitro*** release of Repaglinide from film F_5 in phosphate buffer (pH 7.4).

The release data of repaglinide are processed into graphs (Figures 30 to 39) to understand the linear relationship, i.e., kinetic principles. The data were processed for regression analysis using MS-Excel statistical functions. The parameters and equations are given in the Table 31.

Table 31: Comparison of orders of *in vitro* release Repaglinide from films F_1 to F_5

Film code	***In vitro* release in phosphate buffer pH 6.8 regression equations**	
	Zero order	**First order**
F_1	y = -0.021x + 1.745 R^2 = 0.771	Log y = -2.741x + 89.08 R^2 = 0.89
F_2	y =-0.021x + 1.745 R^2 = 0.771	Log y = -1.972x + 57.62 R^2 = 0.508
F_3	y = -0.057x + 2.109 R^2 = 0.817	Log y = -3.748x + 89.56 R^2 = 0.983
F_4	y = -0.087x + 0.166 R^2 = 0.892	Log y = -5.191x + 93.16 R^2 = 0.945
F_5	y = -0.228x + 2.123 R^2 = 0.919	Log y = -11.60x + 82.61 R^2 = 0.909

A perusal to the Table 31 indicated that the regression values of film F_1 and F_2 are higher with zero order and therefore the release kinetics followed zero order from these two films and films F_3 to F_5 are higher with first order and therefore the release kinetics followed first order from these films.

Release Mechanisms

To study the release mechanisms of repaglinide the data of *in vitro* drug release was verified using Higuchi's model, Korsmeyer-Peppas model, and Hixon-Crowell cube root law models.

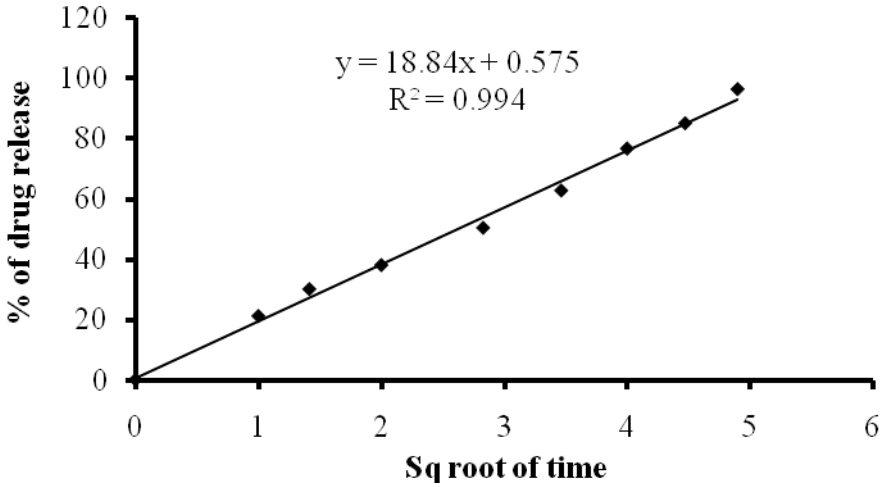


Figure 38:*In vitro* release profile Repaglinide from of F-3 fitted in Higuchi's Plot

In order to explore more precise mechanism of release of repaglinide from in house developed matrix tablets, the dissolution data was also fitted to the well-known exponential equation (Korsmeyer equation) as shown in the Figure 40, which is often used to describe the drug release behaviour from polymeric systems.

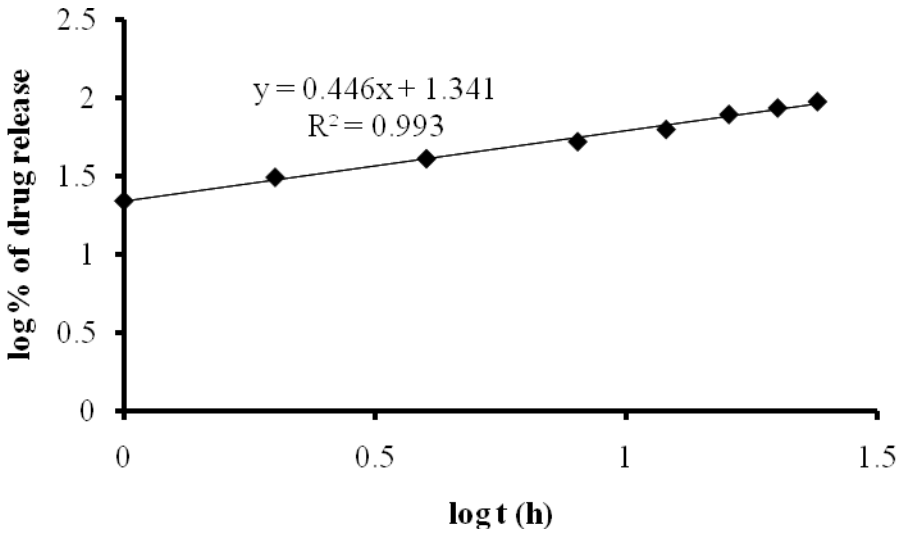


Figure 39: *In vitro* release profile of Repaglinide from F-3 fitted in Korsmeyer's Plot.

Table32: Fitting of the Hixon-Crowell cube root law for *in vitro* release of Repaglinide from F-3

Time in hours	$M_0^{1/3} - M^{1/3}$
0	0.000
1	0.170
2	0.249
4	0.343
8	0.468
12	0.597
16	0.846
20	1.019
24	1.300

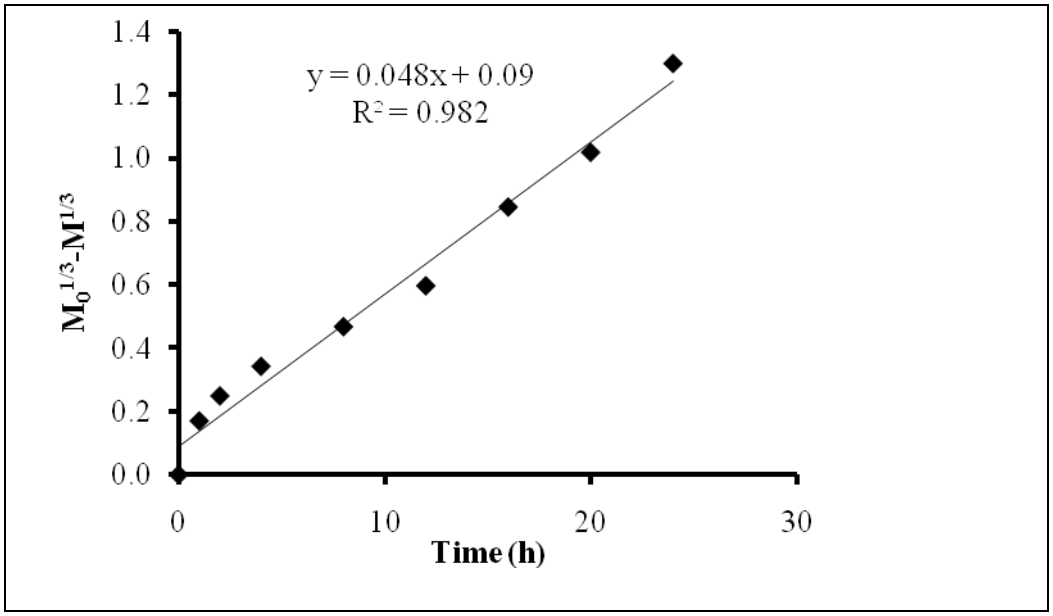


Figure 40: Fitting of the Hixon-Crowell cube root law for *in vitro* release of Repaglinide from F-3

Table 33: Regression equations of *in vitro* release of repaglinide from the film F-3

Formulation	***In vitro* release of repaglinide from the formulation of F-3**		
	Hixon-Crowel model	**Higuchi's model**	**Korsmeyer Peppas model**
F3	y = 0.048x + 0.09 R^2 = 0.982	y = 18.84x + 0.575 R^2 = 0.994	y = 0.446x + 1.341 R^2 = 0.993

Application of Hixon–Crowell cube root law, the equation ($M_0^{1/3} - M^{1/3}$) = kt, provides information about the release mechanism, namely dissolution rate limited. Application of Higuchi's equation ($M = K\,t^{1/2}$) provides information about the release mechanism, namely diffusion rate limited. Korsmeyer-Peppas model indicates that release mechanism is not well known or more than one type of release phenomena could be involved. The 'n' value could be used to characterize different release mechanisms as:

Table 34: Slope of Korsmeyer-Peppas equation and proposed release mechanisms

Slope (*n*)	**Mechanism**
<0.5	Fickian diffusion (Higuchi Matrix)
0.5 <*n*< 1	Non-Fickian diffusion
1	Case II transport

The data of average values were processed as per Hixon-Crowell cube root law and are given in the Tables 32 and the Figure 40. The data of average values were processed as per Higuchi's equation and are represented in the Figure 38. The data of average values were processed as per Korsmeyer-Peppas model and are represented in the Figure 39. The linearity of data for all the models was identified from the Figures. The equations were generated through statistical procedures and reported in the Table 33.

Perusal to the Figure 40 indicates that R^2 values are higher for the Higuchi's model compared to Hixon – Crowell for the F-3. Hence repaglinide release from the tablets of F-3 followed diffusion rate controlled mechanism.

According to Korsmeyer-Peppas model, a value of slope < 0.5 indicates the Fickian diffusion (Higuchi Matrix) and hence release mechanism from F-3 follows Fickian diffusion (Higuchi Matrix).

Stability Studies

Optimized medicated patches were subjected to short term stability testing. Films were placed in a glass beaker lined with aluminum foil and kept in a humidity chamber maintained at 40 $\pm$ 2 ^{o}C and 75 $\pm$ 5% RH for 1 month as per ICH guidelines. Changes in the appearance and drug content of the stored films were investigated after storage. The data presented were the mean of three determinations. Percentage drug present in the films was determined spectrophotometrically and reported in the Table 35 and represented in the Figure 43. Percentage decrease in drug content in all the films was also calculated and reported in the Table 35 and represented in the Figure 41. Perusal to Tables 35, the Figures 41 indicated that the drug loss is less though the films were stored for one month.

The films were also observed for their appearance and texture. These properties did not change in all the films during the period of study.

Table 35: Percentage drug present in repaglinide film F-3

Time (hours)	Zero day	After 30 days
0	0.0	0.0
1	16.01	14.86
2	20.56	19.72
4	28.71	29.02
6	34.39	33.12
8	42.02	41.59
12	56.54	55.32
16	70.32	70.46
20	85.24	84.67
24	98.24	96.48

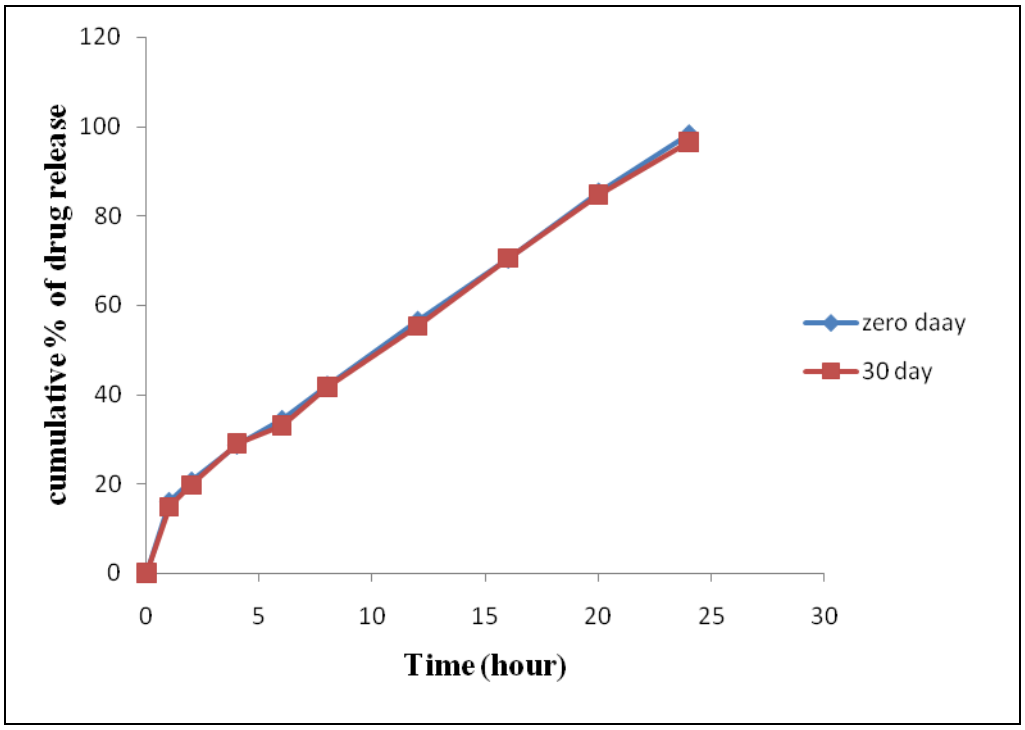


Figure 41: Percentage drug present in repaglinide films F-3 during one month storage.

The percent drug of repaglinide from initial concentration 100% was found to be decreased by 3.52%

From the results obtained and the discussion generated from them, encouraged conclusions were drawn and presented in the next chapter “conclusion”.

SUMMARY

Introduction

Transdermal drug delivery systems (TDDS) are adhesive drug containing devices of defined surface area that delivers predetermined amount of drug to the intact skin at a pre programmed rate. The transdermal delivery has gained importance in recent years. The transdermal drug delivery system has potential advantages of avoiding hepatic first pass metabolism, maintaining constant blood levels for longer period of time resulting in a reduction of dosing frequency, improved bioavailability, decreased gastrointestinal irritation that occur due to local contact with gastric mucosa, and improved patient compliance. Some of the anti diabetic drugs already have been formulated and evaluated as transdermal patches but most of them still been unexplored.

Objectives

In the present study transdermal films of the Repaglinide were prepared using polymers like HPMC K-100 and Eudragit RS100 alone and in different combinations. These transdermal films will be characterized for their physicochemical properties including drug release.

Review of Literature

The chapter 'Literature Review' contained the general concepts of transdermal drug delivery and its applications. Advantages and limitations of drug delivery through transdermal route were listed. Description of the physiology of skin for drug delivery was explained.

Factors affecting transdermal drug delivery system and possible routes for drug transport across the skin layer were discussed. Basic components and modern approaches of transdermal drug delivery systems were described.

Methodology

At the outset, method for estimation of the drug was developed. Repaglinide showed maximum absorption at wavelength 237 nm in phosphate buffer pH 7.4 and 0.1 N HCl solutions. Standard calibration curve obeyed Beer's law at given concentration range of 1µg/ml to 20 µg/ml which one subjected to regression analysis. The value of regression coefficient was found to be 0.997, which showed linear relationship between concentration and absorbance. The FT-IR peak of repaglinide displayed some parent peaks at 400 to 4000 cm^{-1}nm. The physical mixture of drug with selected polymers gave peak which corresponded to the parent peaks of the pure drug which confirmed the compatibility between drug and selected polymers.

The various formulations of films were developed for repaglinide, dose in given area of 15 cm^2 was 10 mg using various bioadhesive and film forming polymers like Ethyl cellulose and HPMC K-100 in single and, dibutyl phthalate.

Physicochemical parameters such as weight uniformity, thickness uniformity, swelling determinations, tensile strength determination, viscosity determination, and folding endurance studies were carried out. In order to know the pattern of release of Repaglinide from patches, *in vitro* release and skin permeation studies were carried out

with dissolution apparatus and diffusion cell using pH 7.4 phosphate buffers as receptor medium, Short-term stability studies were conducted.

Results and Discussion

The results and discussion obtained from different methods of this thesis were described under different tables and graphs. From the results of the drug content determination, it was inferred that there was proper distribution of drug in the films and the deviations were within the acceptable limits. Films exhibited higher tensile strength as the concentration of HPMC was increased. The prepared film exhibited satisfactory physical characteristics such as weight uniformity, thickness uniformity, and folding endurance. Films containing HPMC alone showed higher WVTR as compared to those containing Ethyl cellulose. This may be due to the HPMC which is more hydrophilic than, Ethyl cellulose, which is less permeable to water vapor. Release study of repaglinide films indicated that the drug release from the formulation F-3 showed satisfactorial result. Among all the developed formations, formulation containing HPMC K-100 and Ethyl cellulose, showed maximum rate of drug release of 98.2467 $\pm$ 0.136 within 24 h. Hixon-Crowell cube root law was applied to test the release mechanism. R^2 values are higher for Higuchi's model compared to Hixon – Crowell for the patchF_3. Hence Repaglinide release from the patch F_3 followed diffusion rate controlled mechanism. Repaglinide release from patches F_4, F_5 followed dissolution rate controlled mechanism. The absorption kinetics was studied by regression analysis

(R^2 =0.935). The absorption of repaglinide followed first order. Stability study of formulations showed no significant changes in the drug content as well as physical characteristics of the patches.

Conclusion

From the results obtained and discussion generated there from, encouraged conclusions were drawn and written in chapter. On the basis of the *in vitro* characterization it was concluded that Repaglinide could be administered transdermally through matrix type TDDS developed in our laboratory. Transdermal patches consisting of the bioadhesive polymer HPMC K-100 and rate-controlling polymer of Ethyl cellulose demonstrated sustained and controlled release of the drug across during *in vitro* permeation studies. The drug remained intact and stable in the TDDS during storage, with no significant chemical interaction between the drug and the excipients. Further work is to establish the therapeutic utility of this system by pharmacokinetics and pharmacodynamic studies on human beings.

CONCLUSION

Repaglinide is one of the drugs, which is used for the management of type 2 diabetes. It has only a short half-life of 1- 3 hour and oral bioavailability is about45%. Therefore, the present investigation is concern with the development of the unidirectional transdermal films and to increase the bioavailability of the drug and its half life.

The following conclusions were drawn from results obtained;

1) A suitable method of analysis of repaglinide by UV Spectroscopy was developed. Repaglinide showed maximum absorption at wave length 237 nm in isotonic phosphate buffer (pH 7.4) and 0.1 N HCl solutions. The value of regression coefficient of standard curve was found to be 0.995 which showed linear relationship between concentration and absorbance. Preformulation studies for drug-polymer compatibility by FTIR gave confirmation about their purity and showed no interaction between the drug and selected polymers.

2) Various formulations were developed by using release rate controlling and bioadhesive polymers like Ethyl cellulose and HPMC K-100 in single and combinations by solvent casting methods with incorporation of dibutyl phthalate as plasticizer.

3) Developed transdermal films possessed the required physicochemical properties such as drug content uniformity, swelling index, folding endurance, weight uniformity, thickness

uniformity, tensile strength, and water vapour transmission rate (WVTR).

4) From the results of the drug content determination, it was inferred that there was proper distribution of drug in the films and the deviations were within the acceptable limits.

5) Films exhibited higher tensile strength as the concentration of HPMC was increased.

6) Films containing HPMC alone showed higher WVTR as compared to those containing Ethyl cellulose.

7) Among all the developed formations, formulation containing HPMC, Ethyl cellulose of equal amount F-3 showed maximum rate of drug release of 98.249 $\pm$ 0.135 within 24 h.

8) Hixon-Crowell cube root law was applied to test the release mechanism. R^2 values are higher for Higuchi's model compared to Hixon – Crowell for the patches F_3. Hence repaglinide release from the patch F_3 followed diffusion rate controlled mechanism.

9) Stability study of the formulations showed no significant changes in the drug content as well as physical characteristics of the film.

FUTURE DIRECTION

Long term stability studies of the films.

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